ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

The Merck Fellowship in Analytical Chemistry

THE Board of Directors of the AMERICAN CHEMICAL SOCIETY at its September meeting accepted a proposal that the Society administer a graduate fellowship in analytical chemistry, financed by Merck & Co., Inc. The action of Merck & Co., Inc., and the Board of Directors of the Society opens a new field of service to the chemical profession by the Society, the forerunner, we hope, of many similar proposals. For the first time in the history of the Society, it has undertaken to administer fellowships financed by industrial concerns. It has, of course, financed a number of predoctoral and postdoctoral fellowships from its own funds, and for years has administered a number of medals and awards provided by industry.

In March 1947, we proposed in ANALYTICAL CHEMISTRY a four-point program for improving the profession of analytical chemistry. One of the suggestions was the establishment of a number of postgraduate fellowships. Again, in the April 1948 issue, we stressed the urgent need for support of analytical chemistry by industry, pointing out that industry is finding it increasingly more difficult to obtain personnel capable of initiating, directing, and interpreting research in analytical chemistry, including physicochemical and purely physical methods.

Turning from a specific recommendation for the profession of analytical chemistry to the broad problem of assisting brilliant graduates in all fields of chemistry and chemical engineering, we stated in the August 9, 1948, issue of *Chemical and Engineering News:*

> Today hundreds of scholarships and fellowships are provided for through funds made available by corporations, both large and small. In this regard the record of the chemical industry and industries allied to it is an outstanding one. But much more must be done before we can say truthfully that what is being accomplished is a corrective measure and will offset the efforts of those who would make higher education in the country the full responsibility of the Federal Government.

No strings are attached to the Merck proposal. The fellowship, to be known as the Merck Graduate Fellowship in Analytical Chemistry, will carry $2500 for one year's graduate work. The company has agreed to maintain the fellowship for at least three successive years. A fellow shall be eligible to have his fellowship renewed twice, but no person shall hold the fellowship for longer than a total of three years.

Each applicant or nominee must establish to the satisfaction of the A.C.S. award committee that he is acceptable for graduate work in analytical chemistry at a specified, A.C.S.-approved institution in the United States or Canada and a proposed research and study outline must be submitted to the award committee.

The A.C.S. award committee will award the fellowship to that nominee whom it judges likely to contribute most to the advancement of the theory and practice of the science of analytical chemistry, not merely during the tenure of the fellowship but in his future career. The award committee will decide on the manner of payment of the fellowship money, and on what, if any, reports are to be required of the fellow.

The committees of the Society which administered the predoctoral and postdoctoral fellowships financed by the Society's Educational Fund set a very high standard of performance. We have heard nothing but praise for the manner in which these committees carried on the admittedly difficult task of selecting applicants. The experience in administering the ACS fellowship program should make it relatively simple to develop procedures based on the previous methods of operation.

Our heartiest congratulations and deep-felt thanks to the directors of Merck & Co., Inc., to George Merck who took a very personal interest in the proposal, and to Randolph T. Major and Beverly L. Clarke for active interest and support in bringing the company and the Society together on the fellowship plan.

The action of Merck & Co., Inc., should stimulate other industrial firms to do likewise. Here is a very practical way of demonstrating that private enterprise in the chemical field has a deep and lasting interest in the progress of the chemical profession. Here is an opportunity for industry to assist the youth of America to gain professional training in one of the most challenging professions, as well as to help provide the scientific manpower which it will surely need if the present rate of progress is to be maintained and accelerated as it should be in the future.

Many members of the AMERICAN CHEMICAL SOCIETY are in a position to influence management to offer fellowships similar to the one just presented by Merck & Co., Inc. If someone will but take the initiative, favorable results are inevitable.

(Reprinted from *Chemical and Engineering News*)

BASIC ASPECTS OF X-RAY ABSORPTION

In Quantitative Diffraction Analysis of Powder Mixtures

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The mathematical relationships are developed which are pertinent to the quantitative analysis of powder mixtures for the case of diffraction from the surface of a flat powder specimen. These formulas relate the diffracted intensity to the absorptive properties of the sample. Three important cases are treated: (1) Mixture of n components; absorbing power of the unknown equal to that of the matrix; concentration proportional to intensity. Direct analysis is permitted. (2) Binary mixture; absorbing power of the unknown not equal to that of the diluent; concentration not proportional to intensity. Direct analysis is possible by means of calibration curves prepared from synthetic mixtures. (3) Mixture of n components; absorbing power of the unknown not equal to that of the matrix; general case. Analysis is accomplished by the addition of an internal standard. Concentration is proportional to the ratio of the intensity of a selected reflection from the unknown to the intensity of a reflection from the internal standard.

DURING recent years x-ray diffraction methods have been extensively applied to problems of quantitative analysis. Diffraction methods possess the unique advantage of detecting not only the presence of chemical elements but also their state of chemical combination. Quantitative measurements of greatly improved quality can now be made with the aid of Geiger-counter tubes for receiving the diffracted energy. Finally, particular impetus has been given to the use of quantitative diffraction techniques by the development and widespread commercial distribution of the Norelco Geiger-counter x-ray spectrometer.

In spite of this extensive activity in the field of diffraction analysis, no investigator to date has published a detailed statement of the simple but important mathematical relationships which relate the diffracted intensity to the absorptive properties of the sample and thereby determine the particular procedure that is suitable for the analysis of any given sample. This communication presents these mathematical considerations for the case of diffraction from the surface of a flat powder specimen, the arrangement employed in the Norelco x-ray spectrometer.

The measurement of the absolute intensities of x-rays diffracted by the components of a binary powder mixture has been discussed theoretically by Brentano (*4*, *5*), and he has applied the results to the measurement of atomic scattering factors. Glocker (*8*) and Schäfer (*10*) have shown in a similar manner that the fundamental intensity formulas of Laue can be used as a basis for the quantitative diffraction analysis of binary powder mixtures and alloys. However, they did not extend their mathematical treatment to the point of evolving a systematic practical scheme of analysis.

INTENSITY DIFFRACTED BY ONE COMPONENT OF A POWDER MIXTURE

It will be initially assumed that the sample is a uniform mixture of n components, that the particle size is very small so that extinction and so-called microabsorption effects (*6*) are negligible, and that the thickness of the sample is sufficient to give maximum diffracted intensities.

A satisfactory criterion for the latter condition is that $\mu s \geq 6.4$, μ being the linear absorption coefficient of the sample and s being the maximum path length traversed by the x-rays through the sample (*12*). Applied to the geometrical arrangement of the Geiger-counter x-ray spectrometer, this criterion takes the form

$$t \geq \frac{3.2}{\mu} \times \frac{\rho}{\rho'} \times \sin\theta$$

in which t is the thickness of the powder sample, ρ is the average density of the solid material composing the powder, ρ' is the density of the powder (including the interstices), θ is the Bragg reflection angle, and μ is the mean linear absorption coefficient of the solid material composing the powder.

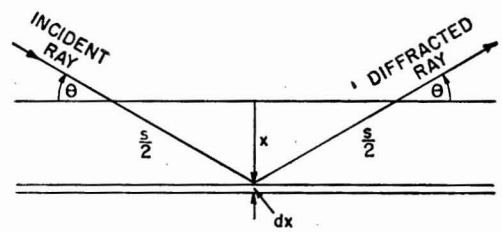

Figure 1. Diffraction from a Layer of Powder at Depth x

Consider such a powder sample consisting of n components and irradiated by an incident x-ray beam of cross-sectional area A which impinges upon the sample at angle θ. Under these conditions the area of sample surface irradiated is $A/\sin\theta$. Referring to Figure 1, consider the diffraction taking place from a layer of thickness dx at depth x. The volume of this layer is

$$dV = \frac{A\,dx}{\sin\theta}$$

or, since $x = \frac{1}{2}\, s \sin\theta$,

$$dV = \frac{1}{2}\, A\,ds \qquad (1)$$

Let us now define $(I_o)_i$ as the intensity diffracted by unit volume of pure ith component at the angle 2θ to the primary beam and under conditions of nonabsorption, and let f_i be the volume fraction occupied by the ith component, neglecting the interstices. The intensity diffracted from element dV by component i of the mixture is then

$$dI_i = \frac{1}{2}\,(I_o)_i f_i A e^{-\mu s} ds$$

and, integrating between the limits $s = 0$ and ∞, we obtain for the total intensity of the diffracted beam from the ith component

$$I_i = \frac{(I_o)_i f_i A}{2\mu} \tag{2}$$

We may now define a constant, $K_i = \frac{1}{2}(I_o)_i A$, which is a function of the nature of the component i and of the geometry of the apparatus. Equation 2 can then be written

$$I_i = K_i \left(\frac{f_i}{\mu}\right) \tag{3}$$

In order to apply Equation 3 to quantitative analysis, it is desirable to express I_i as a function of the weight fraction of the ith component. Let W and V be, respectively, the sample weight and volume (neglecting interstices), while w_i, x_i, ρ_i, and μ_i represent, respectively, the weight, weight fraction, density, and linear absorption coefficient of the ith component. It can then be seen that f_i, the volume fraction of the ith component, is given by

$$f_i = v_i/V = \frac{\frac{w_i}{\rho_i}}{\sum_1^n \frac{w_i}{\rho_i}}$$

Multiplication of the numerator and denominator by $1/W$ gives

$$f_i = \frac{x_i/\rho_i}{\sum_1^n (x_i/\rho_i)} \tag{4}$$

Now the mass absorption coefficient, μ/ρ, of a powder mixture is defined by the expression

$$\mu/\rho = \sum_1^n x_i(\mu_i/\rho_i) \tag{5}$$

But

$$\rho = W/V = W/\sum_1^n v_i$$

$$= \frac{1}{\frac{1}{W}\sum_1^n (w_i/\rho_i)}$$

$$= \frac{1}{\sum_1^n (x_i/\rho_i)}$$

Hence, from Equation 5 we obtain

$$\mu = \rho \sum_1^n x_i(\mu_i/\rho_i)$$

$$= \frac{\sum_1^n x_i(\mu_i/\rho_i)}{\sum_1^n (x_i/\rho_i)} \tag{6}$$

Substituting Equations 4 and 6 in 3, and representing the mass absorption coefficient of the ith component by $\mu_i^* = \mu_i/\rho_i$, we arrive at the relationship

$$I_i = K_i \times \frac{x_i/\rho_i}{\sum_1^n \mu_i^* x_i} \tag{7}$$

Equation 7 can be put into a very useful form by regarding the mixture of n components as if it consisted of just two components, the component to be analyzed for, component 1, and the sum of the other components, which we may call the matrix and refer to by the subscript M. The weight fraction of the matrix is

$$x_M = 1 - x_1 = w_M/W$$

Now the weight fraction of the ith component in the matrix is

$$(x_i)_M = w_i/w_M = \frac{Wx_i}{W(1 - x_1)}$$

$$= \frac{x_i}{1 - x_1} \tag{8}$$

The mass absorption coefficient of the matrix is given by

$$\mu_M^* = \mu_2^*(x_2)_M + \mu_3^*(x_3)_M + \mu_4^*(x_4)_M + \cdots\cdots$$

which by reference to Equation 8 may be written

$$\mu_M^* = \frac{\sum_2^n \mu_i^* x_i}{1 - x_1} \tag{9}$$

In terms of the component to be analyzed for, component 1, Equation 7 becomes

$$I_1 = \frac{K_1 x_1}{\rho_1 \sum_1^n \mu_i^* x_i}$$

$$= \frac{K_1 x_1}{\rho_1 \mu_1^* x_1 + \rho_1 \sum_2^n \mu_i^* x_i}$$

which in view of Equation 9 becomes

$$I_1 = \frac{K_1 x_1}{\rho_1[\mu_1^* x_1 + \mu_M^*(1 - x_1)]}$$

or

$$I_1 = \frac{K_1 x_1}{\rho_1[x_1(\mu_1^* - \mu_M^*) + \mu_M^*]} \tag{10}$$

Equation 10 is the basic relationship underlying quantitative diffraction analysis with the x-ray spectrometer. It relates the intensity of any given diffraction maximum of the unknown component to its concentration in the sample and to the relative mass absorption coefficients of the unknown and matrix.

THREE IMPORTANT ANALYTICAL CASES

According to the number of components and the equality or nonequality of μ_1^* and μ_M^* in Equation 10, three important cases of quantitative analysis arise, each permitting or requiring a particular procedure.

Mixture of n Components; $\mu_1^* = \mu_M^*$; direct analysis. The absorbing power of the unknown equals that of the matrix. Equation 10 reduces to

$$I_1 = \left(\frac{K_1}{\rho_1 \mu_M^*}\right) x_1 = kx_1 \tag{11}$$

which shows that the diffracted intensity is directly proportional to the concentration, allowing direct linear analysis. In practice such cases are relatively rare, the most important being mixtures of the polymorphic crystalline forms of a given compound. More frequently it happens that μ_1^* and μ_M^* are approximately but not precisely equal. In this event the degree of accuracy desired will determine whether or not the linear relationship of Equation 11 can be assumed to apply.

In Figure 2 the validity of Equation 11 is illustrated by means of diffraction data from mixtures of quartz and cristobalite, polymorphs of silica, for which $\mu^* = 34.9$ for CuKα radiation.

Three synthetic mixtures were prepared containing 25, 50, and 75% micronized quartzite admixed with Johns Manville's Celite,

Type I. Celite is a commercial thermal insulating powder which diffraction analysis showed to consist very largely of finely divided α-cristobalite. It also contains a very small amount of clay and possibly a little amorphous silica, but the absorbing powers of these impurities differ but little from that of cristobalite. Each analysis for quartz was performed in triplicate using a Norelco Geiger-counter x-ray spectrometer. The intensity of the 3.33 Å. quartz maximum was measured by manual scanning, taking sufficient counts at each point to keep Geiger-counter statistical errors small. The recorded counts were corrected for the resolving time of the counter. It is seen that the four experimental points lie very close to a straight line as predicted.

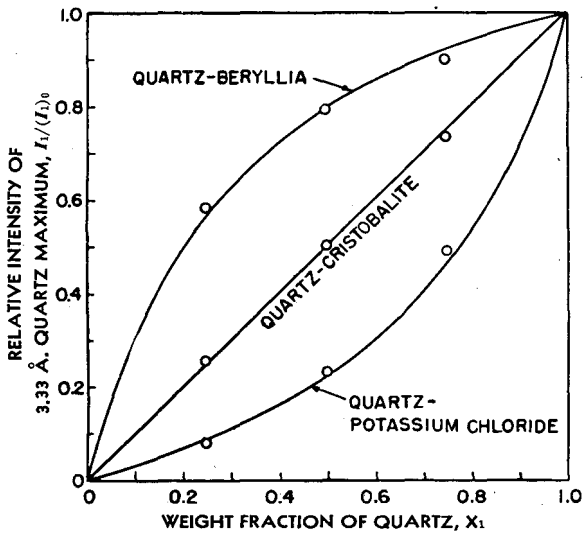

Figure 2. Comparison of Theoretical Intensity–Concentration Curves (Solid Lines) with Experimental Measurements (Open Circles) for Several Binary Mixtures

Mixture of 2 Components; $\mu_1^* \neq \mu_2^*$. The absorbing powers of the unknown and diluent are unequal, so that the intensity-concentration curve is not linear. However, direct analysis is possible by reference to a calibration curve prepared from synthetic mixtures of the two components. An equation giving the theoretical form and position of the analysis curve can be obtained from Equation 10. For the pure first component

$$(I_1)_0 = \frac{K_1}{\rho_1 \mu_1^*}$$

while for a mixture containing a weight fraction x_1 of the first component

$$I_1 = \frac{K_1 x_1}{\rho_1[x_1(\mu_1^* - \mu_2^*) + \mu_2^*]}$$

Division of the second equation by the first gives

$$\frac{I_1}{(I_1)_0} = \frac{x_1 \mu_1^*}{x_1(\mu_1^* - \mu_2^*) + \mu_2^*} \qquad (12)$$

In Figure 2 the upper and lower curves show the agreement between the theoretical values (solid lines) as calculated with Equation 12 and experimental points for mixtures of quartz and beryllium oxide ($\mu_1^* > \mu_2^*$) and mixtures of quartz and potassium chloride ($\mu_1^* < \mu_2^*$). The values of the mass absorption coefficients for CuKα radiation were calculated from the absorption data given in the "Internationale Tabellen zur Bestimmung von Kristallstrukturen" and found to be: BeO 8.6, SiO_2 34.9, and KCl 124. The intensity measurements were made in the manner described above.

Mixture of n Components; $\mu_1^* \neq \mu_M^*$; general case. The absorbing power of the unknown is different from that of the matrix, the latter being unknown as a general rule. In this case the analysis can be performed by the addition of an internal standard. This method has been applied to emission spectrographic analysis for some years (*11*), while more recently it has been utilized in diffraction analysis (*1*, *2*, *3*, *7*, *9*). The latter application has seemed valid on more or less intuitive grounds, but to date no one appears to have investigated its theoretical basis.

Consider, then, the addition of an internal standard, component s, to the sample in known amount. The unknown and internal standard components then occupy the volume fractions f_1' and f_s, the prime being used to distinguish the volume fraction of the unknown component after addition of the internal standard from its fraction in the original sample, f_1.

From Equation 3 we have for these two components,

$$I_1 = K_1 f_1'/\mu \text{ and } I_s = K_s f_s/\mu$$

Dividing I_1 by I_s and substituting for f_1' and f_s from Equation 4, we obtain

$$\frac{I_1}{I_s} = \frac{K_1 \rho_s x_1}{K_s x_s \rho_1}$$

and solution of this equation for x_1' gives

$$x_1' = \frac{K_s \rho_1 x_s}{K_1 \rho_s} \times \frac{I_1}{I_s} = k' \times \frac{I_1}{I_s} \qquad (14)$$

provided x_s is kept constant. But the weight fraction of component 1 in original sample is related to x_1' in the following manner:

$$x_1 = \frac{x_1'}{1 - x_s}$$

When this is combined with Equation 14 we find that

$$x_1 = \frac{k'}{1 - x_s} \times \frac{I_1}{I_s} = k \times \frac{I_1}{I_s} \qquad (15)$$

Equation 15 states that when the internal standard is added in a constant proportion, x_s, the concentration of the unknown component is proportional to the intensity ratio I_1/I_s.

Figure 3 shows the applicability of Equation 15 to the analysis of quartz in powder mixtures using fluorite as internal standard.

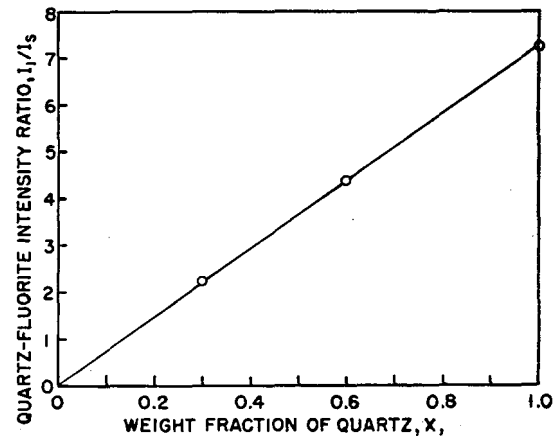

Figure 3. Linearity of Typical Calibration Curve for Quartz Analysis Using Fluorite as Internal Standard

Three synthetic mixtures of micronized quartzite and calcium carbonate were prepared containing 30, 60, and 100% quartz. Fluorite was then added to each in the constant proportion x_s = 0.20. The intensity ratio of the 3.33 Å. quartz and 3.16 Å. fluorite maxima was then measured with the Norelco spectrometer in the manner described earlier. Each plotted point is the average of 10 determinations. It is seen that the experimental results substantiate the linearity between I_1/I_s and x_1 which is predicted by Equation 15.

ACKNOWLEDGMENT

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POLAROGRAPHY

Some Factors Affecting Drop Time

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Data are presented showing the influence of capillary vibration, applied voltage, magnitude of current flowing through the cell, capillary active agents, purity of mercury (including certain amalgams), and stray currents in the constant temperature bath upon the drop time.

IN POLAROGRAPHIC analysis, the concentration of the material sought is determined by the magnitude of the diffusion current produced under the prevailing conditions and the precision is indicated by the degree of reproducibility of the diffusion current in repeated runs. The magnitude of the current, however, depends upon several factors besides the concentration, as shown in the fundamental equation for the dropping mercury electrode, first devised by Ilkovič (*1*):

$$i_d = 605nD^{1/2}Cm^{2/3}t^{1/6}$$

in which n = number of faradays of electricity required per mole
D = diffusion coefficient, sq. cm. per second
C = concentration of substance sought, millimoles per liter
m = mass of mercury flowing, mg. per second
t = drop time, seconds

Of these factors, n and D are inherent in the nature of the material being determined and hence beyond the control of the analyst under the conditions of any given analysis. The mass of mercury is substantially constant for a given capillary operating under a fixed head of mercury and at constant temperature. Variations in diffusion current from one run to another, under otherwise identical conditions, will, therefore, be attributable primarily to irregularities in the drop rate and although time enters the equation to the extent of only the sixth root, a considerable degree of consistency is required if high accuracy is to be attained. Thus, excluding all other errors, a variation of 3% in drop time from one run to another will cause an error of 0.5% in the determination; to attain 1.0% accuracy, the variation in time may not exceed 6.2%.

The investigation described herein was, therefore, undertaken to identify the factors primarily responsible for variation in drop time.

EXPERIMENTAL

A model XX visible recording polarograph, manufactured by the E. H. Sargent Co., Chicago, Ill., was used in this work. The time for full-length chart travel (410 mm.) was 645 seconds.

The polarographic cell was the H-shaped type recommended by Kolthoff and Lingane (*4*), with saturated calomel cell anode, supported in a 30 × 30 cm. (12 × 12 inch) cylindrical Pyrex constant temperature bath maintained at 25° ± 0.05° C. by a Lo-Lag thermostatically controlled immersion heater. The neoprene tube connecting the capillary with the mercury reservoir was provided with a branch leading to a fixed buret for convenient maintenance of the 443-mm. head used throughout the work. Under this pressure, the capillary passed 1.366 mg. per second.

The determinations of drop time were made by establishing the desired conditions and permitting the instrument to run at sensitivity and damping settings to give easily legible pen oscillations per drop, usually for five chart divisions, representing an elapsed time of 322.5 seconds. The oscillations were then counted and the average time per drop was obtained. In some few instances, the average drop time over a definite voltage range was determined by a similar procedure.

Mercury Head. The effect of the mercury pressure was not investigated, as it is fully covered in the literature (*2*, *9*). It has been shown that drop time is an inverse linear function of the head if proper correction is made for the back pressure due to the interfacial tension between the mercury and the solution in which it is dropping.

Vibration. The polarograph itself, containing no galvanometer, is vibration-proof. The effect of vibration on the mercury drops has been noted (*9*, *10*) but no detailed study of it appears to have been made.

At the start of the work, variations of as much as 10% were noted under apparently identical conditions. Vibrations seemed the most likely explanation, although the steel and stone construction bench upon which the water bath and capillary support were mounted did not show enough vibration to interfere with the proper functioning of an analytical balance of 0.05-mg. sensitivity.

A stand was made for the bath assembly consisting of a 35-cm. (14-inch) square of $^5/_8$ inch thick boiler plate, drilled and tapped at the back, left side, and right rear corner for 0.5-inch diameter rods for the support of the capillary and other accessories. Three runs were then made upon 0.1 N potassium chloride containing 1 ml. each of 1 M cadmium chloride and 1% gelatin per 100 ml., at −0.9 volt, a sensitivity of 2–50 (33.9 mm. per microampere) and No. 1 damping. These conditions were chosen to give wide amplitude to the pen oscillations. Varying degrees of vibration insulation were used:

RUN 1. The boiler plate stand was set directly on the bench

and the bath jar thereon with no cushioning at either place. Average drop time, 4.59 seconds.

RUN 2. A 1-inch layer of hair felt was placed under the boiler plate, and 0.5-inch hair felt and a corrugated rubber mat between the plate and jar. Average drop time, 4.89 seconds.

RUN 3. One inch of hair felt, 2 inches of sponge rubber, 0.5 inch of hair felt under the plate; same padding as in run 2 between plate and jar. Average drop time, 4.97 seconds.

To illustrate more clearly the effect of vibration, the total pen travel (from the beginning of one drop to the beginning of the next) for each of 50 drops in each run was measured. The numbers of drops in various travel groups are given in Table I. Inasmuch as the rate of mercury flow was constant throughout all three runs, the pen travel furnishes a measure of individual drop time.

Table I. Effect of Vibration on Drop Time

	Number of Drops		
Pen Travel, Mm.	Run 1	Run 2	Run 3
108–109	1	0	0
109–110	6	0	0
110–111	7	1	0
111–112	12	0	0
112–113	9	8	0
113–114	7	30	0
114–115	6	11	0
115–116	2	0	2
116–117	0	0	18
117–118	0	0	23
118–119	0	0	7
Av. travel	112.0	113.5	117.2

The most satisfactory form of bath agitation was found to be air, led to near the bottom of the jar through a tube 6 mm. in inside diameter with a right-angle bend at the lower end, flowing at the rate of about 5 bubbles per second. The surface waves caused by the air bubbles have no appreciable effect, presumably because of their low frequency, but both air and electric-driven propeller agitators, even when mounted on a separate stand, caused irregularities. Air agitation was used in the above runs.

In this vibration study, the polarograph cells were suspended in the water bath by means of ordinary laboratory clamps mounted on one of the support rods screwed into the boiler plate base. Subsequently, it was found that a Koppers viscometer clamp (Fisher Catalog No. 15-347-165) mounted on the rim of the jar not only is more convenient but tends to reduce vibration still further.

Stray Currents. It was noted that the amplitude of the pen oscillations was somewhat affected by the operation of the immersion heater, confirming a similar observation by Lingane (*6*). Although no concomitant variation in drop time could be detected, irregularity in amplitude is likely to cause error in measurement of wave height in the analysis.

This effect was neutralized by grounding the water by immersing a double strand of No. 16 bare copper wire connected to a water pipe. About 25 grams of sodium chromate were dissolved in the bath to furnish conductance and prevent corrosion of the various metals. The bath was grounded when the vibration study was made, as in all subsequent work.

To avoid a similar effect, the lead wires to the polarographic cell and mercury reservoir should be kept well away from 110-volt alternating current lines to the heater or other apparatus.

Applied Voltage. As the influence of applied potential upon drop time has been extensively studied and reported in the literature (*3, 5, 8*) a detailed discussion here is unnecessary. It was noted, however, that in the voltage range of approximately +0.3 to −0.6 volt, the variation among the results of repeated determinations was about three times as much as at numerically higher voltages, either positive or negative, in either 0.1 *N* potassium chloride or tetramethyl ammonium chloride.

Mercury Purity. In the work reported so far, two kinds of mercury were used: ordinary laboratory grade as received from the chemical supply houses, and the same after having been used for general laboratory purposes and then purified by aeration and washing with dilute nitric acid. No difference was noted in the polarographic functioning of these two.

In the following work, the special thermometer grade, virgin mercury was used in the hope of eliminating the erratic values in the critical voltage range mentioned above, but the data were no more concordant than those yielded by ordinary mercury. Attention was therefore directed to the effects of known added impurities. Liquid amalgams have been used as the dropping electrode (*7, 11*), but their effect upon drop rate has not been specifically studied.

The amalgams were prepared by dissolving the desired metal in ordinary reagent grade mercury, squeezing through chamois leather, and washing with methanol and acetone to remove grease. Metals covering a wide range in the electromotive series were used.

ZINC. A 1% solution was tried first, but even at very low sensitivity (about 0.2 mm. per microampere), maximum damping, and no applied voltage, the pen oscillation per drop was nearly 80 mm. When diluted successively to 0.01 and 0.0001% zinc concentrations, the usual type of curve for drop time *vs.* voltage was obtained but with no improvement in concordance in the critical voltage range. The pen oscillations per drop in the positive voltage range were exceedingly irregular in both shape and amplitude.

TIN. A 0.01% tin amalgam was used. At zero and −0.1 applied voltage, the pen oscillations were so irregular as to defy counting. From −0.3 to −1.8 volts, the oscillations were regular, but the data when plotted were exceedingly erratic. Under positive voltage, the drops became so rapid, several per second, that the machine could not follow them.

COPPER. The copper concentration in this amalgam was about 0.01%. Again, at 0.0 and −0.1 volt the drops were too erratic to count. From −0.3 to −1.8 volts, a fairly smooth time-voltage curve was obtained. In the positive range, the drop times increased greatly, reaching 12.2 seconds at 0.9 volt, the highest voltage tested.

SILVER. A 0.13% solution was used, this being about the maximum solubility of silver in mercury at 25° C. Once again, the usual type of time-voltage curve was obtained, with a high degree of discordance among the points in the critical range.

GOLD. A 0.1% amalgam in thermometer grade mercury was used, representing approximate saturation. Pen oscillations were regular throughout the negative voltage range and but slightly irregular in the positive, but the data were distinctly more erratic than with untreated mercury.

Table II. Effect of Current on Drop Time

Current, microamp.	0.57	13.8	24.5	48.5	68.5	116.0
Drop time, sec.	4.69	5.03	5.05	5.04	5.12	5.16

These results indicate that from about −0.6 to +0.3 volt is a region of somewhat unstable equilibrium not produced by the presence or absence of impurities in the mercury but inherent in the polarographic system. Increasing concentrations of gelatin up to 1%, although progressively decreasing the average drop time, do not eliminate the inconsistencies in this region.

Cleanliness of Capillary Tip. Minute traces of oil on the flat face of the capillary produce irregular drops. This can be avoided by keeping the tip submerged in ethanol when not in use. This bath should be placed at such a height that the alcohol does not back up into the capillary nor mercury flow therefrom at an appreciable rate. Droplets of mercury or air bubbles adhering to the flat surface may also cause trouble, although this was not specifically investigated.

Current. To determine the effect of the current flowing through the cell upon the drop time, runs were made in 0.1 *N* potassium chloride to which were added successive amounts of cadmium chloride solution to give the desired currents. The voltage was held constant at −0.9, which is above the reduction potential of cadmium.

Capillary Active Agents. Runs were made on 0.1 *N* solutions of potassium chloride and tetramethylammonium chloride over the voltage range 0 to −1.2. In the first run of each series, no

capillary active agent was used; in the second, 0.0001% of methyl red was used, and in the third, 0.01% gelatin, these concentrations being those most generally used in polarographic work. The average drop times were found to be 5.09, 4.94, and 4.63 seconds, respectively, in potassium chloride and 5.00, 5.02, and 4.65 in tetramethylammonium chloride.

Some of the published polarographic analytical methods have directed that the gelatin solution be prepared fresh daily but have given no reason. A brief study was therefore made of the effect of the age of the gelatin solution.

Two 1% solutions of Knox's No. 1 cooking gelatin were prepared, to one of which 1 ml. of chloroform per 100 ml. was added. These were stored at room temperature for 9 days and then used, along with a freshly prepared solution for comparison, in the polarography of a dimethylnitrosamine solution in 0.2 *N* hydrochloric acid, 1 ml. of gelatin solution being added per 100 ml. The drop time was determined over the voltage range −0.6 to −1.2. Wave heights and half-wave potentials are also included in Table III.

Table III. Effect of Stale Gelatin Solutions

Gelatin	Drop Time, Sec.	Wave Height, Mm.	$E_{1/2}$, Volt
Fresh	4.40	206.5	−0.95
Stale	4.35	199.0	−1.02
Stale + $CHCl_3$	4.45	205.5	−0.95

Similar results were obtained with gelatin solutions 3 and 5 days old. Although the effect upon drop time is slight, both wave height and reduction potential were seriously affected in the absence of chloroform preservative. In addition to these effects, the appearance of the polarograms is different with stale, unpreserved gelatin in the following principal respects:

In the rapidly rising portion of the curve, the pen progresses more or less uniformly instead of in re-entrant waves.
Pen oscillations in the plateau are of distinctly less magnitude.
The angle of the plateau to the horizontal is increased.

Summarizing these effects, it appears as though, through bacterial or mold action, compounds have been formed in the gelatin which act as electrical insulators, coating each mercury drop as it forms with a high resistance layer.

Supporting Electrolyte. The nature and concentration of the electrolyte as well as the solvent used all have some effect upon the drop rate, resulting from changes in the tension at the mercury-solution interface.

The effect of concentration is usually small. Thus, with potassium chloride +0.01% gelatin at −0.9 volt, the average values given in Table IV were found.

Table IV. Effect of Electrolyte Concentration

Concn., *N*	0.0001	0.001	0.01	0.10	1.0	4.0
Drop time, sec.	5.10	4.89	4.74	4.87	4.91	5.10

In 0.1 *M* concentration and 0.01% gelatin, sodium and ammonium hydroxide, acetic and hydrochloric acids, and potassium and tetramethylammonium chlorides all gave substantially the same drop time.

The effect of solvent is appreciable, 0.1 *N* potassium chloride plus 0.01% gelatin in water giving a drop time of 4.93 seconds; in 50% methanol, 4.70; in 50% dimethylformamide, 4.57.

Summarizing these data, it is evident that in order to make reasonably accurate calculations by means of the Ilkovič equation, the drop time should be known under the exact conditions of the analysis rather than under some arbitrarily chosen conditions.

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TITANIUM

Polarographic Determination in Clays and Clay Products

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A polarographic method for the determination of titanium in clays, leached clay residues, sulfite-sulfate leach liquors, precipitated basic aluminum sulfate, and alumina has been developed. This procedure can be adapted to the analysis of other titaniferous material. The analyses were conducted in a supporting electrolyte of 1.0 *N* sulfuric acid, saturated with sodium oxalate and 8% urea for maximum suppression. The effects of the concentration of acid, urea, and aluminum, and titanium, and the iron-titanium ratio upon the diffusion current and half-wave potential of titanium *vs.* the saturated calomel electrode have been determined. A comparison of the standard titanium curve and calculations based on the Ilkovič equation is presented.

THROUGHOUT the investigation of the problem of extracting alumina from Pacific northwest clays using a combined sulfite-sulfate process (*5*), frequent analyses for titania were required. By following the titania composition of the unleached and leached clays, the leach efficiency can be calculated. Titania was chosen as the basis for calculation of the chemical balance because it is the clay constituent least attacked during the leaching process. Less than 2% of the total titania in the system is found in the leach liquor.

A procedure for the polarographic determination of titanium in titaniferous material using a 0.1 *N* sulfuric acid supporting electrolyte was described by Zeltzer (*8*) and a chloride system was discussed by Strubl (*7*). A routine check of Zeltzer's method showed an inadequate wave form for titanium (Figure 1, .1)

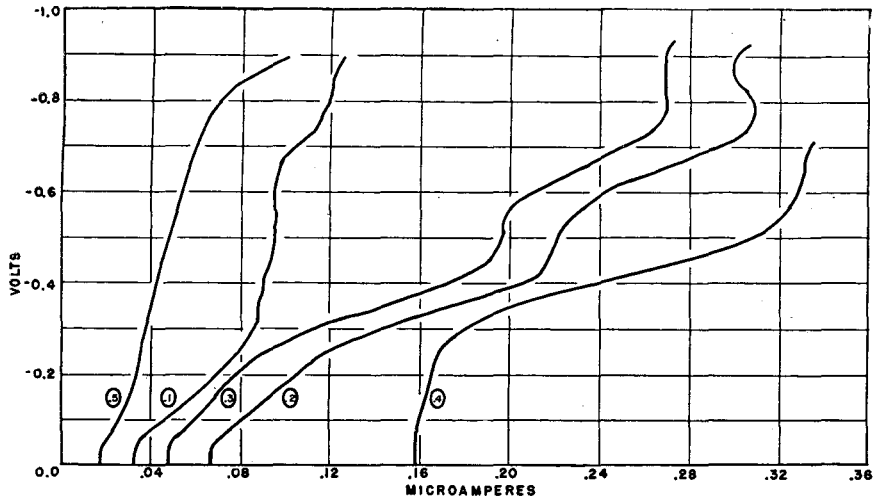

Figure 1. Typical Curves

	Titanium	Saturated with
.1	0.37 millimolar	Sodium oxalate
.2	0.37 millimolar	Sodium oxalate and 3.2% urea
.3	0.37 millimolar	Sodium oxalate and 8.0% urea
.4	0.37 millimolar	Sodium oxalate and 8.0% urea
.5	Residual current	

Supporting electrolyte, 0.1 *N* H_2SO_4

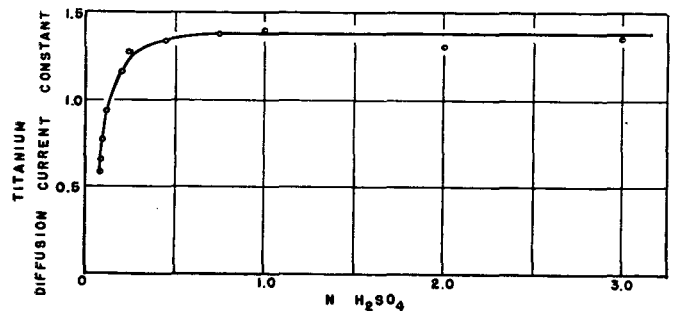

Figure 2. Effect of Sulfuric Acid Concentration on Diffusion Current Constant

which varied with the acid concentration. The curves in Figure 1 originate at an initial current of 0.0 with the exception of .4, which was displaced depending upon the iron concentration. All samples contained 0.37 millimolar titanium.

PRELIMINARY INVESTIGATION

A sulfate supporting electrolyte was chosen because the special systems under investigation contained sulfate as the principal anion. A check of the effect of various complexing agents was undertaken in an endeavor to improve the wave form shown in Figure 1, .1. Of the various complexing ions used—tartrate, citrate, peroxide, and oxalate—only a solution saturated with sodium oxalate showed a sufficiently well formed wave (Figure 1, .2).

The wave thus obtained exhibited a strong maximum and the usual maximum suppressant materials, gelatin and glue, removed the maximum, but simultaneously reduced the diffusion current to such a point that the removal of the maximum was accompanied by disappearance of the titanium wave. According to Kolthoff and Lingane (*2*), organic indicators are also used to reduce a maximum. Attempts to remove the maximum in the titanium curve with sodium indigo disulfonate, bromophenol blue, methyl red, etc., were not successful. Upon the addition of urea to the system, however, the maximum was completely removed without affecting the diffusion current of the titanium (Figure 1, .3 and .4).

The preliminary investigation was carried out using 0.1 *N* sulfuric acid as supporting electrolyte as outlined by Zeltzer. The results obtained were not reproducible; therefore, an investigation of the effect of the acid concentration upon the diffusion current constant was undertaken. In each case the solution was saturated with sodium oxalate and sufficient urea was added to remove the maximum (Figure 2). The curve shows the sensitivity of the diffusion current in acid concentrations from 0.1 *N* to 0.75 *N*. A supporting electrolyte of 1.0 *N* sulfuric acid, saturated with sodium oxalate, lies sufficiently in the straight-line portion of the curve of diffusion current constant against acid concentration to yield consistent results. The concentration of urea required was found to be a function of the sulfuric acid concentration. A 1.0 *N* solution required 8% urea by weight to suppress the maximum.

It was determined that the half-wave potential *vs.* the saturated calomel electrode for both titanium and iron became less negative as the normality of the sulfuric acid supporting electrolyte increased. This relationship for titanium is shown in Figure 3. At an acid concentration approximately 1.0 *N*, the ferric-ferrous wave starts at zero applied e.m.f. Lingane (*4*) had previously reported this fact.

Polarograms of a series of titanium samples from 0.063 to 9.26 millimolar titanium were recorded and the resulting half-wave potentials of titanium *vs.* the saturated calomel electrode were plotted in Figure 4. The half-wave potential of titanium, using three different concentrations of titanium (0.4, 0.6, and 1.61 millimolar), became more negative as the concentration of iron increased, as shown in Figure 5.

On the basis of the preliminary investigation it was concluded that the ideal system for the polarographic determination of titanium would consist of a supporting electrolyte of 1.0 *N* sulfuric acid, saturated with sodium oxalate, 8% urea, titanium between 0.063 and 0.10 millimolar, and an iron-titanium ratio of 10 or below. By use of the compensation method of measuring diffusion currents (*2*), a higher ratio might be tolerated. An empirical correction chart (Figure 7), prepared for use in analyzing sulfite-

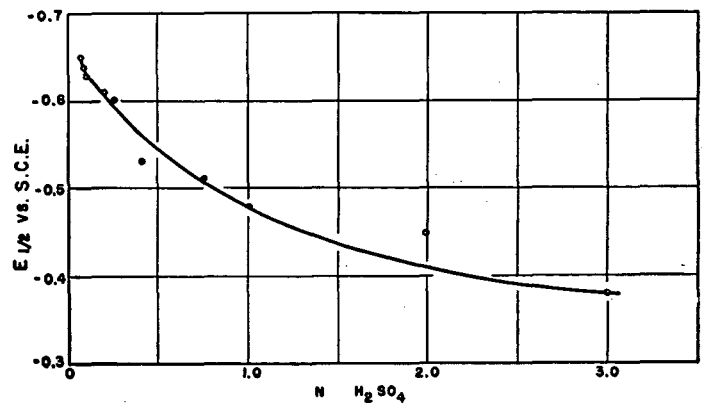

Figure 3. Effect of Sulfuric Acid Concentration on Half-Wave Potential of Titanium

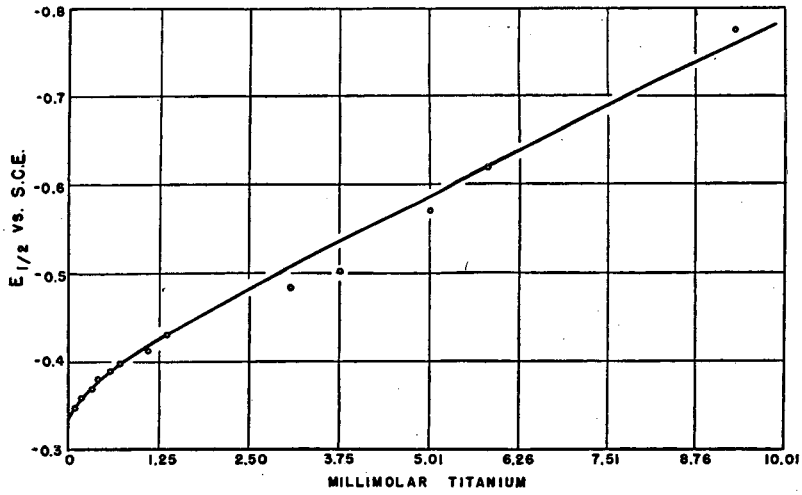

Figure 4. Effect of Titanium Concentration on Half-Wave Potential of Titanium

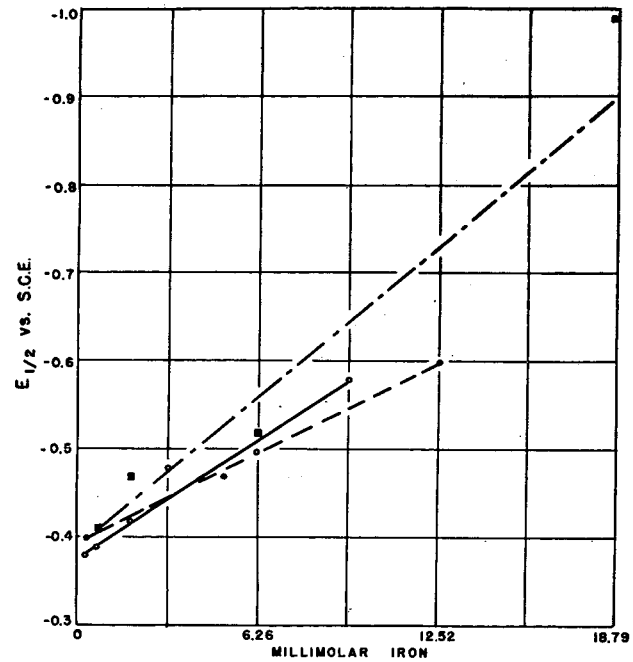

Figure 5. Effect of Iron Concentration on Half-Wave Potential of Titanium

sulfate leach liquors, relates the increase in half-wave potential of the titanium due to the presence of a high iron concentration (Figure 5) with the decrease in diffusion current produced by that high iron concentration (Figure 6).

In Figure 7 line AB corresponds to an iron-free titanium solution, the parallel lines CD, EF, and GH correspond to constant concentrations of titanium with varying amounts of iron increasing from D to C, etc. To illustrate the use of the empirical correction chart suppose, for example, that the polarogram of titanium solution containing iron shows a half-wave potential and a diffusion current as indicated by the point M. Extrapolate from M to the base line AB parallel to CD. The point M' obtained in this fashion gives immediately the diffusion current of the iron-free titanium solution. Because of the recorder lag experienced with the author's instrument, each investigator will have to work out his own correction for the effect of iron.

Strubl (*6*) used hydroxylamine in the presence of excess iron to reduce the iron and thus enable a small amount of titanium to be determined, claiming no harmful effects. It was found that the titanium diffusion current varied as the concentration of hydroxylamine was changed.

Upon calculation of titanium content of samples containing a high aluminum-titanium ratio, it was discovered that the titanium calculated was approximately 30% lower than the colorimetric value. A sample containing 0.63 millimolar titanium and 98 millimolar aluminum was analyzed in the usual manner and a second diffusion current constant was calculated and used with success in the presence of a high aluminum-titanium ratio (Table II).

APPARATUS AND MATERIALS

A Leeds & Northrup Electrochemograph was employed in this study. The reference electrode was a Beckman No. 9740 saturated calomel electrode. The commercial nitrogen was passed over copper wire heated to 450° C. to remove traces of oxygen, through a sintered-glass diffusion disk in a column of water immersed in a constant temperature bath at 25° C. to saturate with water vapor, and then to the polarographic cell to displace dissolved oxygen. The capillary characteristics are shown in Table I.

Table I. Capillary Characteristics

		Capillary 1	Capillary 2	Capillary 2 with High Aluminum Concn.
Height of mercury column, mm.	h	254	254	254
Temperature, ° C.	T	25 ± 0.2	25° ± 0.2	25° ± 0.2
Drop time, sec.	t	4.0	3.8	3.8
Mass of mercury, grams	m	1.917	1.688	1.688
Concentration of titanium (millimolar)	C	0.659	0.628	0.628
Diffusion current, microamperes	i_d	1.641	1.413	0.983
Diffusion current constant (calcd.)	I	1.280	1.272	0.882

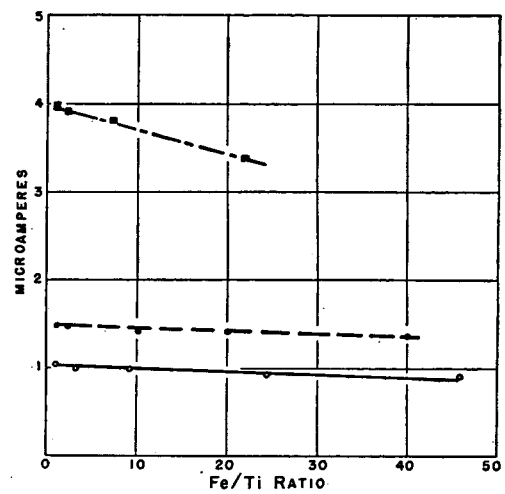

Figure 6. Effect of Iron-Titanium Ratio on Diffusion Current of Titanium

All reagents used were analytical reagent grade. The urea was added either in the solid form or as a solution, 100 ml. of which contained 25 grams. Thus each milliliter of urea solution was equivalent to a 1% addition by weight when 25-ml. volumetric flasks were employed for solution preparation.

ANALYTICAL PROCEDURES

Standard Curve. The standard curve for titanium was obtained by recording the polarograms of a series of titanium samples from standard titanium sulfate solutions.

A stock solution containing 21.24 millimolar titanium was diluted to yield a series of solutions containing (1) 1.022 millimolar, (2) 3.15 millimolar, and (3) 8.57 millimolar titanium. The titania content of the stock solution was checked gravimetrically and the dilute standard solutions were checked colorimetrically. The sulfuric acid concentration of all standard solutions was adjusted to 1.0 N.

The required amounts of titanium were measured into 25-ml. volumetric flasks, the calculated amount of sulfuric acid was added to bring the final concentration to 1.0 ± 0.05 N with respect to sulfuric acid, 8 ml. of urea solution or 2.0 grams of urea crystals were added, and the solutions were made up to volume. A portion of each solution was placed in the cell, the solution was saturated with sodium oxalate, and the oxygen was removed by bubbling for 20 minutes with purified nitrogen. The polarogram of the solution was recorded from 0.0 to −0.8 volt at 25° C.

Figure 8 shows the millimoles per liter of titanium in all standard solutions analyzed and the microamperes of diffusion current obtained. A comparison of the results obtained in this manner with computations employing the Ilkovič equation was made (Table II).

Table II. Determination of Titanium

Sample Leach Residue	Colorimetric, %	Polarograph, % Calcd.	Polarograph, % Graph
27-6	3.58	3.57[a,b]	3.58[a]
2-12	3.53	3.54	3.56
3-10	3.18	3.21	3.22
3-9	3.28	3.26	3.26
4-8	3.08	3.24	3.25
4-6	3.28	3.29	3.30
5-4	3.25	3.29	3.30
16-10	2.65	2.65[c]	
16-9	3.28	3.37	
16-8	3.30	3.28	
16-7	3.08	3.09	
16-6	3.25	3.21	
16-5	2.91	2.91	
16-4	2.92	2.81	
16-3	2.95	2.96	
16-2	2.85	2.90	
16-1	2.78	2.71	
Raw clay	2.71	2.65	
Basic aluminum sulfate	0.117	0.118[d]	
Leach liquor	0.713 millimolar	0.724 millimolar[d]	

[a] Electrode 1. [b] I = 1.28. [c] Electrode 2. [d] I = 0.882.

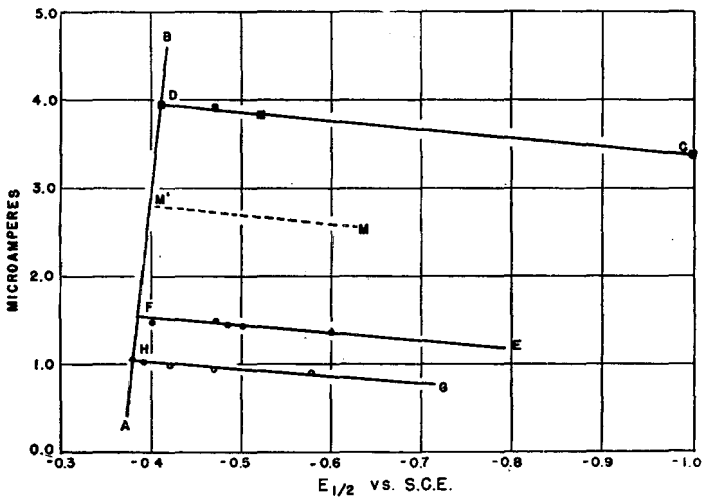

Figure 7. Titanium Half-Wave Potential *vs.* Titanium Diffusion Current

Empirical correction chart for effect of iron on titanium diffusion current

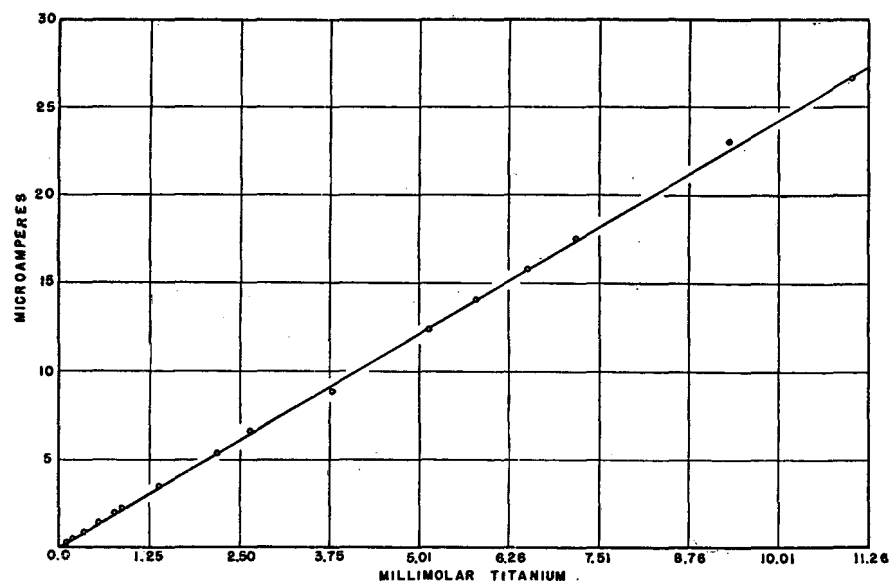

Figure 8. Titanium Concentration *vs.* Diffusion Current

Leach Residues. The leach residues to be analyzed are first dried, ground, mixed, and split into appropriate sized samples. A 0.2000-gram sample (titania content approximately 7 mg.) is weighed out and fused with 3 grams of potassium hydrogen sulfate. The fusion is carried out in a 200-ml. tall-form beaker over a Geauque burner. The sample is cooled, leached with dilute sulfuric acid, and transferred to a 100-ml. volumetric flask. A 10-ml. aliquot is placed in a 25-ml. volumetric flask along with 8 ml. of urea solution and 2.4 ml. of 10 N sulfuric acid (to bring the final acidity to approximately 1.0 N) and made up to volume. A portion of the sample is placed in the polarographic cell and saturated with sodium oxalate, the dissolved oxygen is removed with purified nitrogen, and the polarogram is recorded from 0.0 to −0.8 volt.

Original Clay and Calcined Alumina. Titanium is determined in a manner similar to that used in the leach residues, with an appropriate adjustment on the sample weight to yield a final titanium concentration between 0.025 and 0.10 millimolar.

Leach Liquor. A 10-ml. sample of the liquor (titanium content approximately 0.63 millimolar) is taken and evaporated with 25 ml. of 1.0 N sulfuric acid to volatilize the sulfur dioxide. The sample is cooled and transferred to a 50-ml. volumetric flask. A 10-ml. aliquot is placed in a 25-ml. volumetric flask and 8 ml. of urea solution and the required amount of sulfuric acid are added. A portion of the solution is taken and record made as in the previous determinations.

RESULTS

The Ilkovič equation (*1*) expresses the diffusion current completely and quantitatively by the following relationship:

$$i_d = K_n D^{1/2} C m^{2/3} t^{1/6}$$

in which i_d is the diffusion current in microamperes, n is the number of electron equivalents per molar unit of the electrode reaction, D is the diffusion coefficient (square centimeters per second) of the reducible or oxidizable substance, C is its concentration in millimoles per liter, m is the rate of mercury flow from the dropping electrode in milligrams per second, and t is the drop time in seconds. The linear dependence of i_d and $Cm^{2/3}t^{1/6}$ has been established (*2*).

The terms k, n, and D are independent of characteristics of the dropping mercury capillary, and are determinable experimentally as $i_d/Cm^{2/3}t^{1/6}$, which is referred to as the diffusion current constant, I (*3*).

The concentrations of titanium in the samples analyzed were determined by standard titanium curve, or calculated from the relation $C = i_d/Im^{2/3}t^{1/6}$. The use of this relation, with the previously determined value of the diffusion current constant and m and t values allows an interchange of electrodes with the elimination of a standard curve for each electrode (*3*).

The essential data for comparison of diffusion current values is given in Table I. Diffusion current measurements were made after correction for the residual current.

Titanium was determined on a series of column leach residues, leach liquors, raw clay, and basic aluminum sulfate by both colorimetric and polarographic means (Table II).

The observed diffusion currents obtained during the preparation of the standard titanium curve and the diffusion current constant indicate the linear relation of the system over a large range of concentrations (Figure 8).

CONCLUSIONS

By means of the procedure described it is possible to determine titanium in a variety of materials. The optimum range in concentration for quantitative work is from 0.063 to 0.10 millimolar titanium with an ultimate range from about 0.012 to 6.3 millimolar. The effect of the sulfuric acid concentration upon the titanium diffusion current has been determined and a satisfactory maximum suppressant described. The maximum allowable iron-titanium ratio, without resort to the compensation method of measuring diffusion currents, has been determined. An empirical correction chart has been drawn to correct for the higher iron-titanium ratio in sulfite-sulfate leach liquors. Diffusion current constants were determined for the titanium oxalate complex in a 1.0 N sulfuric acid supporting electrolyte and for the complex in the presence of a high concentration of alumina.

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Polarographic Determination of Iron

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Iron can be determined polarographically by measuring the height of the anodic wave of iron (II) in a slightly acidic oxalate supporting electrolyte. Two methods for the preparation of the solution are presented. With small percentages of iron, the method is inherently capable of much greater accuracy than classical volumetric or gravimetric procedures.

PREVIOUSLY published procedures—e.g. (*3*)—for the polarographic determination of iron have depended on the measurement of the height of the wave produced by reduction of ferric iron in acid solution to the ferrous state. By such methods, ferric iron cannot be distinguished (*21*) from other ions, such as copper (II), chromate, and vanadate, which are also reduced from the beginning of the polarogram. In this paper there are presented two methods of analysis based on a wave having a height uniquely dependent on the concentration of iron.

EXPERIMENTAL

Diffusion current measurements were made with a manual instrument in which a Type K potentiometer was used to measure the potential drop across a 10,000-ohm standard resistance in series with the dropping electrode.

An H-cell (*13*) provided with a saturated calomel reference electrode, and a vertical stand tube connected to an automatic m-measuring device (*10*), completed the polarographic assembly.

A water thermostat was used to maintain a temperature of 25.00° ± 0.01° C. Dissolved air was removed from solution by tank nitrogen freed from oxygen by passage through a train of vanadous sulfate solutions (*20*).

All pH measurements were made with a Beckman glass electrode meter adjusted to read a pH of 3.57 with saturated potassium bitartrate (*7*).

PROCEDURE

Lingane (*12*) reported that an acidic citrate supporting electrolyte of pH between 5 and 7 appeared to offer more promise for use in the determination of iron than any other of the media he investigated. In 0.5 M sodium citrate, pH 6, in the presence of 0.005% gelatin, his polarograms show an excellently defined anodic wave with 2 millimolar ferrous iron. Attempts were made to apply this wave to the determination of iron, but it was found that, with very small concentrations of iron, the wave is exceedingly ill defined, and that the gelatin caused serious abnormalities in the wave height, so that, as shown in Table I, the diffusion current is only approximately proportional to the concentration of ferrous iron. Similar irregularities have previously been remarked (*16*) when gelatin was used to suppress maxima of anodic waves, and it is suggested that the phenomenon may be general in all such cases. In the absence of gelatin, the anodic wave of ferrous iron in 1 M citrate solutions is ill defined at any pH between 2 and 11.

None of these difficulties arises, however, when an acidic oxalate supporting electrolyte, of pH about 5, is used: the anodic wave given by the oxidation of the oxalatoferrous complex to the

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Table I. Diffusion Current Constant of Ferrous Iron in 1 *M* Sodium Citrate, pH 6.7

Successive additions of air-free ferrous ammonium sulfate solution were made to 49.96 ml. of a 1 *M* sodium citrate solution containing 0.008% gelatin and previously titrated to pH 6.7 with dilute sulfuric acid. $E_{d.e.}$ = 0.00 volt *vs.* the saturated calomel electrode. t = 4.36 seconds, $m^{2/3}t^{1/6}$ = 1.693 mg.$^{2/3}$sec.$^{-1/2}$. Diffusion currents have been corrected for the residual current.

Ferrous Iron, Millimolars	Diffusion Current, Microamperes	id/C
0.493	−0.644	−1.306
0.588	−0.758	−1.289
0.678	−0.884	−1.304
0.768	−1.002	−1.305
0.857	−1.108	−1.293
0.944	−1.236	−1.309
1.029	−1.348	−1.310
1.113	−1.461	−1.313
1.196	−1.580	−1.311
1.278	−1.694	−1.326
1.358	−1.809	−1.332
1.437	−1.905	−1.326
1.512	−2.018	−1.335
1.588	−2.106	−1.326

Mean id/C = −1.315 ± 0.012 (mean deviation).
Mean $id/Cm^{2/3}t^{1/6}$ = −0.778 ± 0.007 (mean deviation).

oxalatoferric complex displays no maximum in the absence of gelatin. With the exception of copper (II), which must be removed, no other ion stable in dilute acid solution containing sulfur dioxide gives a wave that will interfere. The iron wave is so well defined—e.g., (*6*, p. 169)—that deviations of even 50 millivolts in either direction from the potential (−0.05 volt *vs.* the saturated calomel electrode), at which measurement of the diffusion current is recommended here, will not sensibly affect the values secured.

Lingane's data (*12*) indicate that the half-wave potential of the reversible ferrous-ferric couple in 0.2 *M* oxalate is, very closely, $E_{1/2}$ = −0.212−0.0076 pH at pH values between 3.7 and 5.3, and the values are not significantly different in 1 *M* potassium oxalate (*8*). Under the conditions used in this investigation, the diffusion current constant, $i_d/Cm^{2/3}t^{1/6}$, was found to be −1.373 ± 0.002 for the oxidation of the ferrous complex with the particular capillary used (Table II).

Table II. Diffusion Current Constant of Ferrous Iron in 1 *M* Potassium Oxalate, pH 4.8

Successive aliquots of air-free ferrous ammonium sulfate solution were added to 49.96 ml. of a 1 *M* potassium oxalate solution previously titrated to pH 4.8 with dilute sulfuric acid. $E_{d.e.}$ = −0.05 volt *vs.* the saturated calomel electrode. t = 3.33 seconds, $m^{2/3}t^{1/6}$ = 1.647 mg.$^{2/3}$sec.$^{-1/2}$. Diffusion currents have been corrected for the residual current.

Ferrous Iron, Millimolars	Diffusion Current, Microamperes	id/C
0.396	−0.893	−2.255
0.524	−1.180	−2.252
0.649	−1.467	−2.260
0.772	−1.741	−2.255
0.884	−2.004	−2.267
1.011	−2.290	−2.265
1.130	−2.556	−2.262
1.242	−2.802	−2.256
1.356	−3.075	−2.268
1.465	−3.309	−2.259
1.575	−3.552	−2.255
1.682	−3.809	−2.265
1.785	−4.032	−2.259

Mean id/C = −2.260 ± 0.004 (mean deviation).
Mean $id/Cm^{2/3}t^{1/6}$ = −1.373 ± 0.002 (mean deviation).

The use of the diffusion current constant has been recommended (*9*, *11*) as making possible direct comparison of data secured by different laboratories. This constant is derived from the Ilkovič equation (*4*, *5*, *6*, *19*) $i_d = 605\ nD^{1/2}Cm^{2/3}t^{1/6}$ by separating the terms which, at constant temperature, are independent of the experimental conditions, giving $I = i_d/Cm^{2/3}t^{1/6} = 605\ n\ D^{1/2}$. The factors that limit the reproducibility of this constant have been studied by Lingane and Loveridge (*14*, *15*, *18*), by Buckley and Taylor (*1*), and by the writer. Because of these factors, the constants quoted in this paper may be found to vary by several per cent from capillary to capillary, and accuracy such as is reported here cannot be expected, in the present state of diffusion current theory, unless a direct calibration (*23*) is made with the capillary to be used in subsequent measurements. Nevertheless, the constants in this paper are considered to render possible, without calibration using standard solutions, analyses to an accuracy that has generally been considered satisfactory in polarographic measurements.

Two rapid and accurate methods of analysis based on this wave are given below. The second is preferred because of its greater versatility, but the first is somewhat more rapid and convenient when the sample contains only traces of elements whose oxalates are insoluble at pH 5. This first method is not otherwise applicable because of the danger that ferrous iron will be occluded in the oxalate precipitates.

Table III. Polarographic Determination of Iron in Bureau of Standards Samples

Sample and Bureau of Standards Certificate Values	Iron Found, % Method I	Iron Found, % Method II
Ferrovanadium 61 Fe 52.8, V 31.15, Mn 3.57, P 2.43, S 0.003, Si 7.78, Al 0.02, Cu 0.29, Ni 1.33, Cr 0.52, Mo 0.72, As 0.004, U 0.01, Ti 0.23	.....	52.65 ± 0.07
Calcium molybdate 71 Fe 1.92, Mo 35.3, Ti 0.06 (CaO 22.0, SiO_2 17.5, SO_3 0.40, Na_2O 1.5, K_2O 0.45)	.....	1.917 ± 0.011
Light aluminum casting alloy 86 Fe 1.52, Cu 7.66, Zn 1.50, Si 0.35, Mg 0.022, Mn 0.01, Ti 0.017, Zr 0.007 (provisional)	.....	1.526 ± 0.003
Manganese bronze 62b Fe 0.82, Cu 57.39, Zn 37.97, Mn 1.29, Al 0.97, Sn 0.96, Pb 0.28, Ni 0.27, Si 0.048, Sb 0.005, As 0.005, Ag 0.005	0.817 ± 0.002	0.819 ± 0.003
Sheet brass 37a Fe 0.27, Cu 70.34, Zn 27.07, Sn 0.97, Pb 0.99, Ni 0.39	0.2748 ± 0.0006	0.2741 ± 0.0005
Sheet brass 37b Fe 0.21, Cu 70.36, Zn 27.09, Sn 0.99, Pb 0.90, Ni 0.45	0.2037 ± 0.0007	0.2008 ± 0.0006
Cast bronze 52 Fe 0.12, Cu 88.33, Zn 1.89, Sn 7.90, Pb 1.52, Ni 0.13, Sb 0.16	.....	0.1246 ± 0.0009

Method I. A sample containing not less than 10 mg. of iron is weighed out into a 125-ml. Kjeldahl flask and 10 to 20 ml. of concentrated hydrochloric acid are added. The mixture is heated gently, with addition of a barely sufficient volume of 1 to 1 nitric acid, until solution is complete, then 40 ml. of water and 20 grams of metallic silver, prepared as for a Walden silver reductor (*22*, *24*), are added. The solution is boiled vigorously for about 5 minutes, then 5 grams more of silver are added and the boiling is continued for a minute or two longer. The silver chloride, excess silver, and precipitated metallic copper are filtered off through a fine quantitative filter paper, and the solution and washings are caught in a calibrated 100-ml. volumetric flask. One gram of anhydrous sodium sulfite is added and the solution is diluted to the mark.

Method II. The sample, dissolved as in Method I, is evaporated to near dryness, 20 ml. of concentrated hydrochloric acid are added, and the evaporation is repeated. The residue is taken up in 25 ml. of 7.75 *M* hydrochloric acid, transferred to a 100-ml. separatory funnel, and extracted three times in rapid succession with 30- to 40-ml. portions of peroxide-free isopropyl ether (*2*, *17*). The combined ethereal phases are treated with 10 ml. of water, to which saturated sodium carbonate solution is added until evolution of carbon dioxide has ceased and the hydrous ferric oxide in the aqueous layer has coagulated. Further re-extractions with dilute sodium carbonate are indicated if the ethereal phase is not then colorless, which may be the case if much molybdenum is

present. The aqueous phases are combined in a calibrated 100-ml. volumetric flask, the precipitate is just dissolved by addition of concentrated hydrochloric acid, 1 gram of sodium sulfite is added, and the solution is diluted to the mark.

The solution finally secured by either method is analyzed by adding a known aliquot to a known volume of air-free 1 M potassium oxalate, previously titrated to pH 4.8 with dilute sulfuric acid, in a polarographic cell. The anodic diffusion current at $E_{d.e.} = -0.05$ volt *vs.* a saturated calomel electrode is related to the concentration of ferrous iron in the solution thus composited by the equation $-i_d = (1.373\ m^{2/3}t^{1/6})\ C$.

Because of the ease with which the oxalatoferrous complex may be air-oxidized, it is convenient to make additions of the sample solution from a microburet whose tip projects through the stopper of the cell and whose stopcock is provided with a side tube through which purified nitrogen can be bubbled to free the solution from dissolved air. This can easily be done while nitrogen is being passed through the supporting electrolyte, and several measurements of $i_d/[V/(V+v)]$, where V is the volume of sample solution added to v ml. of supporting electrolyte, can then be made in a matter of a few minutes.

RESULTS

Each of the analyses of a variety of National Bureau of Standards samples listed in Table III is based on the mean of ten such independent measurements. In every case the polarographic result agrees with the bureau's value to well within the probable uncertainty of the latter.

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Electrolytic and Polarographic Determination of Zinc in Thorium

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Methods for the determination of zinc in thorium-zinc alloys ranging from 20 p.p.m. to 100% zinc are described. An electrolytic method is used for alloys that contain more than 1% zinc. In this procedure the thorium is complexed with citrate, so that it does not interfere with the deposition of zinc. For thorium containing less than 1% zinc, a polarographic method is used. In this method the thorium is complexed with sulfosalicylate. The solution used contains 0.02% gelatin to suppress the zinc maximum and has a pH of 8.5. In both electrolytic and polarographic methods nitrate interferes.

IN THE metallurgical and metallographic research on thorium metal, a need arose for the determination of zinc in thorium-zinc alloys which ranged from 25% down to a few parts per million of zinc. The only previous paper found by the authors that reports a determination of zinc in the presence of thorium is that of Lang (*14*), who used an iodometric method. This method was tried by the authors, and was found to give good results even in the presence of 200 times as much thorium as zinc. However, if equipment is available, the electrolytic method is more convenient for occasional analysis of these alloys, as it does not require the preparation of standard solutions. The lower limit of the range of both the electrolytic and the iodometric methods is about 0.5% zinc in thorium. Smaller amounts of zinc are most conveniently determined by a polarographic method.

REAGENTS

Acetone, reagent grade. Ammonium hydroxide, 15 N, reagent grade. Gelatin, reagent grade. Solution, 0.5%.

Hydrochloric acid, 12 N, reagent grade. Methyl red, 0.2% in alcohol. Perchloric acid, 70%, reagent grade. Sodium citrate, reagent grade. Solution, 60% by weight.

Sodium fluosilicate, reagent grade. Sodium hydroxide, reagent grade. Sulfosalicylic acid, Eastman (676) white label grade. Solution, 2 M.

Thorium, recast, containing less than 10 p.p.m. of zinc. Solution prepared by dissolving a weighed sample in hydrochloric acid containing a little sodium fluosilicate to speed up the reaction.

Zinc, 99.99+% pure, from Bunker Hill and Sullivan Mining and Concentrating Co., San Francisco, Calif. Solution prepared and standardized by dissolving a weighed amount of zinc in dilute hydrochloric acid, diluting to volume, and weighing the solution. The standardization was checked by weighing as sulfate the zinc from a weighed portion of the solution (*22*).

ELECTROLYTIC METHOD

Because of the rather high negative reduction potential of zinc (−0.76 volt), special care must be used in its electrolytic determination. Oxidizing agents stronger than hydrogen ion must be

carefully excluded. Nitrate especially is reported to interfere, causing low results if present in more than trace amounts (*2*, *21*, *23*, *27*). The pH must be kept high to prevent the zinc deposit from going into solution as fast as it is deposited. Determinations have been made in buffered acetate, citrate, and formate solutions (pH of 4.5 to 5) (*1*, *9*, *16*, *21*, *27*, *28*), in buffered ammonia solutions (pH of about 9) (*9*, *24*, *26*), and concentrated sodium hydroxide solutions (*3*, *9*, *18*).

Thorium has a more negative reduction potential than zinc and cannot be deposited from aqueous solutions. However, thorium hydroxide is precipitated at a pH of 3.5, so that if the pH is increased enough to allow quantitative deposition of zinc, a gelatinous precipitate of thorium hydroxide is present in the solution, which collects on the electrodes and causes erratic results. This difficulty is eliminated by using citrate, which forms a strong soluble complex ion with thorium in neutral and alkaline solutions, and also serves as the buffering agent.

Apparatus. Two 6-volt storage batteries in series were used to provide the power for electrolysis. The applied voltage for each electrolytic cell was controlled by a 100-ohm rheostat used as a potential divider. The electrodes, obtained from E. H. Sargent and Co., were made of platinum gauze, the anode acting as stirrer for the solution (see *23*, p. 10, Figure 2, for illustration of the electrodes). The cathodes were plated with copper before the electroposition of zinc (*27*).

Experimental Work. The difficulty in the accurate electrolytic determination of zinc in the presence of thorium is threefold. First, of course, is the reactivity of the zinc metal toward acids and oxidizing agents. It was found in approximate agreement with Winchester and Yntema (*27*) that to prevent low results from this source the pH had to be 5 or above. The second source of error is the tendency of the zinc to form a black powdery deposit. However, with careful handling the amount rubbed off is negligible, and the results were found to be high when the deposit was powdery, probably because of inclusion of foreign material. Addition of acetone as brightener made the deposit much better, and the results, which were high without acetone under the same conditions, were more nearly correct. Cooling the beakers in an ice-water bath during the electrolysis did not seem to help the results (*2*). In accord with the observations of Winchester and Yntema (*27*), 1 ampere was found to be the optimum current to obtain complete deposition, yet not give a powdery form to the deposit. However, there is almost always a small amount of powdery deposit, and the results tend to be high.

The third source of difficulty is the presence of thorium, which tends to cause erratic results if it precipitates. This factor was eliminated by the proper choice of acid for dissolving the alloy and of complexing agent for thorium. Chloride did not interfere with the determination of zinc when no thorium was present. However, with thorium a persistent precipitate formed when the chloride solution was treated with sodium citrate and made alkaline to methyl red with sodium hydroxide. Nitric acid was not used because of its oxidizing properties. Sulfuric acid was not usable because the insoluble hydrate of thorium sulfate, which precipitates on heating, causes the solution to spatter. The alloys are not appreciably decomposed by sulfuric acid. Perchlorate was found not to interfere in any way with this determination. However, perchloric acid does not attack alloys low in zinc very rapidly. For this reason the alloys are first treated with hydrochloric acid which reacts vigorously with the metal, and when this first reaction has ceased, the perchloric acid is added and the solution is evaporated to perchloric acid fumes. Sodium citrate was found to be the best complexing agent for the thorium. Ammonium salts gave low results because of their complexing action on the zinc ion. Tartrate formed a heavy precipitate in the presence of acetone, and sulfosalicylate formed a precipitate with thorium that did not dissolve appreciably in boiling.

Platinum cathodes must be plated with copper before use in the electrodeposition of zinc (*2*).

Good results were obtained with up to 2 grams of thorium in a solution containing 15% sodium citrate. However, when 4 grams were used and concentration of citrate was doubled, the zinc results were low, presumably because of the complexing action of this large amount of citrate on the zinc. This limits the range of the determination to about 0.5% zinc at the lower limit, as 10 to 15 mg. of zinc are the minimum that can be determined accurately. The maximum amount of zinc that can be plated quantitatively is about 0.2 gram.

Procedure. A sample of thorium-zinc alloy containing 15 to 200 mg. of zinc and not more than 3 grams of thorium is placed in a tall-form beaker and covered with water, and 15 ml. of concentrated hydrochloric acid are added cautiously. After the vigorous action of the acid with the alloy has ceased, 5 ml. of 70% perchloric acid are added and the solution is evaporated to perchloric acid fumes on a hot plate. The sides of the beaker are washed down and the solution is again evaporated to fumes. Fifty milliliters of water are added, after the solution has cooled, followed by 25 ml. of 60% sodium citrate. The solution is made just alkaline to methyl red with sodium hydroxide and is warmed until the initial precipitate is completely dissolved. The solution is then diluted to 100 ml. and 15 ml. of acetone are added. The electrolysis is carried out with a previously copper-plated platinum cathode, with an anode stirrer at a current of 1 ampere for 1.5 hours. The electrode is washed with water as it is slowly raised from the solution, with the current still on. It is then washed successively in alcohol and ether, dried for 1 minute at 110 °C. and weighed.

Table I lists the results obtained on synthetic mixtures using the above procedure. In trials 9 to 12 the electrolyte contained 30 grams of sodium citrate instead of 15 grams. These results were all low.

POLAROGRAPHIC METHOD

A number of papers have been published on the polarographic determination of zinc. None describes a method for zinc in which thorium was present, although several describe polarographic determinations of zinc in the presence of the more common complexing agents. Oxalate was used by Prajzler (*19*) to complex nickel and by Knanishu and Rice (*10*) to complex iron so that these elements would not interfere with the wave for zinc. Hybbinette (*8*) used citrate to complex cobalt and nickel, Vyakhirev (*25*) used thiocyanate, and Kolthoff and Matsuyama (*12*) used a citrate-thiocyanate mixture for iron, copper, lead, and nickel. Ensslin, Dreyer, and Abraham (*6*), DePaola (*5*), and Lingane (*17*) used a tartrate electrolyte for zinc. None has used sulfosalicylate, however. There have been very few papers concerning the polarography of thorium (*7*, *13*, *15*).

Apparatus. A Heyrovský polarograph, Model XII (Sargent), was used for this work. The cells, supplied with the instrument, were 10 ml. Erlenmeyer flasks, each with a side arm for the anode lead, one for flushing the sample with nitrogen, and one for the escape of this nitrogen. For some of these experiments a mercury pool was used, but for most of them a saturated calomel half-cell of the conventional type was used as a reference electrode. Contact of the saturated calomel electrode with the solution in the cell was made through the stopper with a side arm containing a

Table I. Electrolytic Separation of Zinc from Thorium

Trial	Th Present *Grams*	Zn Taken *Gram*	Zn Found *Gram*	Error *Mg.*
1	1	0.0885	0.0888	+0.3
2	1	0.0951	0.0954	+0.3
3	1	0.0997	0.0998	+0.1
4	1	0.1021	0.1024	+0.3
5	1	0.0879	0.0884	+0.5
6	1	0.1052	0.1057	+0.5
7	2	0.0811	0.0813	+0.2
8	2	0.1033	0.1033	±0.0
9	4	0.0585	0.0570	−1.5
10	4	0.0529	0.0513	−1.6
11	4	0.0551	0.0544	−0.7
12	4	0.0578	0.0565	−1.3

Table II. Polarographic Determination of Zinc in Thorium without Complexing Agent[a]

Trail	Th Concn.	Zn Concn. Taken	Zn Concn. Found		Error
	Mole/l.	*Millimoles/ l.*	*Millimoles/ l.*	*Microamp.*	%
1	0.039	0.052	0.053	0.34	+1.9
2	0.039	0.143	0.125	0.80	−12.5
3	0.039	1.62	1.37	8.8	−14.7
4	0.004	3.89	4.0	25.5	+2.8
5	0.039	7.85	7.95	51.0	+1.3

[a] Temperature, 28° C.

Table III. Polarographic Determination of Zinc in Thorium

Sample No.	Zn Taken	Diffusion Current	Zn Found	Error
	P.p.m.	*Microamperes*	*P.p.m.*	*P.p.m.*
1	19	0.115	18	−1
		0.118	19	±0
2	20	0.124	20	±0
3	41	0.304	49	+8
4	42	0.231	37	−5
5	59	0.368	59	±0
6	62	0.359	57	−5
		0.399	64	+2
7	79	0.525	84	+5
8	101	0.557	89	−12
9	101	0.662	106	+5
		0.683	109	+8
10	123	0.766	123	±0
11	141	0.870	139	−2

saturated potassium chloride–agar mixture. The mercury pool was used as the anode only in those cases where the supporting electrolyte was 0.1 *N* potassium chloride, so that the electrode would be equivalent to 0.1 *N* calomel half-cell. The dropping mercury electrode was that supplied with the apparatus. The temperature was kept at 25° ± 0.5° in most of these experiments by a constant temperature bath.

Experimental Work. The half-wave potential for zinc in 0.1 *N* potassium chloride supporting electrolyte was found to be −1.09 volts *vs.* the mercury pool, and −1.02 volts *vs.* the saturated calomel electrode, which is in fair agreement with −0.995 volt *vs.* the saturated calomel electrode as reported in the literature (*11*). In agreement with the observation of Langer (*15*), it was found that thorium in the presence of sufficient excess of nitrate gave a break, the step height of which was proportional to the thorium concentration. This break falls at the same position as the zinc break—i.e., −1.1 volts *vs.* the mercury pool. In the absence of nitrate, thorium gave no break at all; nitrate must therefore be excluded from the solutions. Zinc cannot be determined polarographically at a pH lower than 1.5, because its break is masked by that of hydrogen (*11*, *20*). As thorium hydroxide begins to precipitate at pH 3.5, the pH range is limited to acid solutions, unless the thorium is complexed so that it is not precipitated. A good wave for zinc in the presence of thorium ion was obtained at pH 3. The concentration was proportional to the current over a range from 0.00005 to 0.008 *M*. Results with samples corresponding to alloys varying from 0.038 to 28.2% zinc are given in Table II.

The galvanometer sensitivity was varied from $^1/_5$ to $^1/_{200}$ maximum sensitivity, by means of the Ayrton shunt. If several known samples in the one sensitivity range to be used were employed for the calibration, the precision would probably be better.

It is generally more convenient to complex the thorium, and determine the zinc at a higher pH, as the procedure is less time-consuming and some of the more easily reduced impurities, such as iron and nickel, are prevented from interfering. Several complexing agents were tried. Oxalate worked well for thorium concentrations on the order of 1 millimole per liter, but for greater concentrations the thorium precipitated. Zinc is also somewhat complexed, as its half-wave potential is shifted to more negative values in oxalate solutions. No zinc wave was obtained in a citrate solution. This is in accord with the observations of Kolthoff and Matsuyama (*12*), that in a citrate solution no zinc wave is obtained unless aluminum is present. No break was found for zinc in a tartrate solution, in contrast with the results of Lingane and others (*5*, *6*, *17*).

When sulfosalicylate was used to complex thorium, the zinc break occurred at −1.05 volts *vs.* the saturated calomel electrode essentially the same half-wave potential as zinc in potassium chloride, thus showing that zinc was not measurably complexed by the sulfosalicylate. Sodium and potassium sulfosalicylate are soluble in concentrations up to about 0.5 *M* only, so that in the determination of small amounts of zinc, in which a large sample of thorium is used, it was necessary to use ammonium sulfosalicylate as electrolyte. In the presence of ammonium ion the half-wave potential of zinc was fairly constant at −1.05 volts *vs.* saturated calomel electrode up to a pH of about 5. Above this pH the half-wave potential became more negative because of the complexing action of ammonia on zinc ion. In the sodium sulfosalicylate solutions and ammonium sulfosalicylate solutions at pH 7 or below, the nitrate break does not mask the zinc wave, so that it does not interfere. The nitrate break is evidently shifted to a more negative value by the complexing action of sulfosalicylate on the thorium. In alkaline ammonium sulfosalicylate solutions, however, the half-wave potential of the zinc is negative enough so that nitrate again interferes.

Determinations can be made at any pH between 5 and 10, but the pH for a series of determinations must be kept approximately the same to obtain reproducible half-wave potentials and wave heights. A pH of 8.5 ± 0.2 was used by the authors. At a pH of 3 to 4, concentrated solutions of thorium sulfosalicylate had a tendency to set in a gel that was slowly redissolved at higher or lower pH values. For this reason ammonia should be added rapidly to acid solutions and the solutions quickly stirred in order to pass this pH as rapidly as possible.

In only a few cases was a maximum noticeable in the zinc wave. However, the results were generally more consistent when 0.02% gelatin was added as maximum suppressor.

Results listed in Table III were obtained using the recommended procedure. The concentrations are given in parts of zinc per million parts of thorium. As in all cases the thorium concentration was 100 grams per liter (0.43 *M*); the concentration of zinc in the solutions may be obtained by dividing the given values by 10. The factor relating the concentration to the current is in this case 160 p.p.m. per microampere. This, of course, would vary with the concentration of thorium, the characteristics of the capillary of the mercury electrode, and composition of the supporting electrolyte.

Procedure. A sample of thorium weighing 10 grams or less (depending on the zinc content of the metal) is weighed into a 600-ml. beaker and covered with water and 50 ml. of concentrated hydrochloric acid are carefully added. After the vigorous reaction has ceased, about 1 mg. of sodium fluosilicate is added, and the solution is boiled until the black residue has dissolved. The solution is evaporated to 20 ml. and 25 ml. of 2 *M* sulfosalicylic acid and 4 ml. of 0.5% gelatin solution are added. Twenty milliliters of ammonia are then added and the solution is stirred until all the precipitate has dissolved. The pH is adjusted to 8.5 ± 0.2, and the solution is transferred to a 100-ml. volumetric flask and diluted to volume. About 10 ml. of the solution are placed in the cell and flushed 15 minutes with nitrogen to remove dissolved oxygen, and a polarogram of the solution is recorded. The instrument is best calibrated by the standard addition method (*4*). A known amount of pure zinc is added to another sample of the same size, and this sample is treated in the same manner as the first. The concentration of zinc added divided by the difference in the diffusion currents of the two polarograms gives the factor by which the diffusion current of the unknown is multiplied to obtain the unknown zinc concentration.

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Spectrophotometric Determination of Iodine

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A reproducible, sensitive spectrophotometric method for estimation of the iodine content of inorganic and organic materials containing iodine in the range of 1 to 14 micrograms adapts the use of chromium trioxide as an oxidant with distillation of the iodine reduced from the pentoxide in the presence of phosphorous acid. Oxidation of the distillate with permanganate and color development of the starch-iodine chromogen in the presence of potassium iodide give a stable color. The percentage transmittance of this solution is measured with a spectrophotometer using a wave length of 575 mu. Analyses of various materials show that with duplicate samples a replication within 2.5% can be attained with this procedure.

A METHOD for the accurate determination of minute quantities of iodine in rocks, soils, waters, and plant and animal tissues is essential for many investigations.

A review of the pertinent literature revealed that three recent methods appeared to have the required degree of sensitivity, accuracy, and reproducibility. The chromium trioxide oxidation procedure of Matthews, Curtis, and Brode (*9*) seemed to meet requirements more nearly than either of the other two methods (*3, 16*). Salter and McKay (*14*) have discussed difficulties encountered with the catalytic (*3*) procedures, and the titration procedure in which Matthews, Curtis, and Brode (*9*) use 0.0002 *N* thiosulfate is subject to serious error because of the indistinct end point for microquantities of iodine.

Rylich (*13*), Cizek (*4*), Orlemann and Kolthoff (*11*), and Evans, Hanson, and Glasoe (*5*) have developed polarographic procedures, but these have not been applied to routine estimations of iodine in soils and biological materials.

Accordingly, a substitute procedure by which the titration of the liberated iodine could be eliminated was sought. A colorimetric procedure adopting the starch-iodine chromogen was investigated. This chromogen was found to obey Beer's law, and optimum concentration of starch, potassium iodide, and sulfuric acid produced a stable blue color which can be compared with a standard in a visual colorimeter. However, the use of a spectrophotometer permits detection and accurate determination of smaller quantities of iodine.

REAGENTS AND SPECIAL APPARATUS USED IN PROCEDURE

Sulfuric acid, 1.4 *N*, 1.0 *N*, and 0.1 *N*.
Saturated chromium trioxide solution.
Phosphorous acid, 30%.
Sodium hydroxide, 1.0 *N*.
Saturated potassium permanganate solution.
Sodium nitrite, 1.5 *M* (0.104 gram per ml. of solution).
Urea solution, 5 *M* (0.3 gram per ml. of solution).

Arrowroot starch solution. Two grams of starch are ground to a thin paste with a few milliliters of water and slowly poured with constant stirring into 800 ml. of boiling water. The solution is boiled for 15 minutes and 1 gram of salicylic acid is added after cooling. This solution should be freshly prepared about every 2 weeks. This preparation is similar to that used by Nichols (*10*).

Potassium iodide solution containing 0.5 gram per 3.0 ml., prepared just previous to using.

Standard iodine solution, 0.1308 gram of potassium iodide diluted to 1 liter. When 10 ml. are diluted to 1 liter, each milliliter contains 1 microgram of iodine.

Iodine distillation microapparatus described by Matthews, Curtis, and Brode (*9*). (It may be obtained from Leonard Glass Works, Columbus, Ohio, and Harshaw Scientific Co., Cincinnati, Ohio.)

Coleman Universal Spectrophotometer Model 11, with PC4 filter and optically matched cuvettes that give a 40-mm. light path.

EXPERIMENTAL WORK

A solution containing 100 micrograms of iodine was oxidized to iodate ions, using the procedure given below under permanganate oxidation. The oxidized solution was neutralized with

sodium hydroxide and diluted to 100 ml., and 5-ml. aliquots were used in the following experiments:

Spectral Transmittance Curve. A 5.0-ml. aliquot was diluted to approximately 25 ml. in a 50-ml. glass-stoppered volumetric flask, and the following reagents were added: 3.0 ml. of 0.1 *N* sulfuric acid, 3.0 ml. of potassium iodide, and 5.0 ml. of starch solution. The solution was diluted to volume and completely mixed, and after 30 minutes a spectral transmittance curve was made by plotting per cent transmittance (per cent *T*) against wave length. Distilled water was used in the reference cell and the PC4 filter was used in front of the photoelectric cell of the instrument. The cell length used was 40 mm. Maximum absorption for this complex chromogen was found at 575 mu.

Stability of Color. An aliquot containing 5.0 micrograms of oxidized iodine, 3.0 ml. of 0.1 *N* sulfuric acid, 3.0 ml. of potassium iodide, and 5.0 ml. of starch was diluted to 50.0 ml. This solution was well mixed and read immediately at 575 mu and at short intervals during a 90-minute period. Distilled water was used in the reference cell. The color was fully developed at the end of 20 minutes and was stable throughout the 90-minute period studied.

Optimum Concentrations of Reagents. Efforts were made to increase the sensitivity of the method by finding the optimum concentration of each reagent. The amount of each reagent was varied individually in the procedure for stability of color. It was found that maximum color was produced when 4.0 ml. of 0.1 *N* sulfuric acid were used. When potassium iodide was varied from 0.2 to 1.4%, 1% potassium iodide produced the most intense color. The starch was varied from 1 to 9 ml.; 4.0 ml. gave maximum intensity. (Turbidity necessitates use of equal volumes of starch in both cells of the spectrophotometer.) An equal volume of arrowroot starch solution was diluted to 50 ml. for each concentration of starch and used in the reference cell of the instrument. This procedure is similar to the technique used by Reder (*12*) in ascorbic acid determinations.

It was concluded from these experiments that maximum color development is reached at 25 minutes; that the color is stable for 90 minutes; and that optimum concentration of 0.1 *N* sulfuric acid is 4.0 ml., and of potassium iodide 1% of the total volume, in the presence of 4.0 ml. of starch solution.

Standard Curve. Aliquots of the diluted standard solution of potassium iodide containing 1.0 to 14.0 micrograms of iodine were treated as described below under procedure. The concentration was plotted against the logarithm of the per cent transmittance to prepare the working curve.

The curve plotted in this manner is a straight line, confirming the work of Turner (*15*) that the color produced is proportional to the concentration of iodine. Turner included data that showed that temperatures from 4° to 70° C. have no apparent effect on color development. The range of color of the chromogen that can be measured with the spectrophotometer under the conditions outlined is equivalent to 1 to 14 micrograms of iodine per 50 ml. of color solution; transmittance at 1 microgram is near 91% and at 14 micrograms, 10%, depending upon the operator and the instrument used. Further study is needed to establish the procedure in determining iodine in quantities less than 1.0 microgram.

ANALYTICAL PROCEDURE

Chromium Trioxide Digestion. To 1 to 5 grams of sample are added 10 to 30 ml. of chromium trioxide solution and after the initial reaction has subsided, 5 ml. of concentrated sulfuric acid are added for each milliliter of chromium trioxide solution used. [Card (*2*), Graham (*6*), and Alwens and Jonas (*1*) have discussed the irritating effects of chromic acid fumes on the respiratory system. For safety, digestion should be done in a fume hood.] This addition should be made dropwise at first but may be made in larger amounts after the vigorous portion of the reaction is over. An automatic buret attached to the acid bottle simplifies the addition.

After the addition of sulfuric acid the mixture is heated rapidly to 220° C. and maintained at that temperature for 5 minutes to decompose excess chromium trioxide. The flask is rotated to remove any adhering precipitate of chromium trioxide. The solution is cooled to 100° C., 50 ml. of distilled water are added, the contents of the flask are completely mixed, and the flask is connected to the distilling apparatus.

Distillation. A 50-ml. beaker containing 1 ml. of 1 *N* sodium hydroxide is placed under the condenser stem, so that the tip of the condenser is immersed in the solution. The contents of the beaker are heated to boiling, and 10 to 15 ml. of 30% phosphorous acid are added dropwise through the entry tube of the distilling head. Approximately 40 ml. of distilled water are collected, evaporated to about 15-ml. volume, and oxidized in the following manner, as suggested by Groak (*7*).

Permanganate Oxidation. To the boiling distillate are added 1 or 2 drops of a saturated permanganate solution and heating is continued for 5 minutes. The solution is acidified with 1 ml. of 1.4 *N* sulfuric acid (1 ml. of 1 *N* acid to neutralize the sodium hydroxide and 4 ml. of 0.1 *N* sulfuric acid for optimum acidity). At this point, if decoloration of the permanganate occurs, more should be added immediately. After 2 minutes the solution is completely decolorized by adding 1.5 *M* sodium nitrite dropwise at 15-second intervals, directly to the solution and not down the side of the beaker. Boiling is continued for 5 minutes and 4 or 5 drops of 5 *M* urea solution are added to decompose any excess nitrous acid. Spattering of the mixture of permanganate, manganese dioxide, and nitrous acid is likely at this point and is to be avoided. The sides of the beaker are washed down with distilled water.

The contents of the beaker must be agitated after each addition of distilled water to mix the solution completely and to remove material adhering to the beaker walls. Heating is continued for 5 minutes, and the solution is cooled to room temperature and transferred to a 50-ml. glass-stoppered volumetric flask. Then 3.0 ml. of potassium iodide and 4.0 ml. of starch solution are added, and the solution is diluted to the volume, and mixed well. A blank, consisting of 4.0 ml. of 0.1 *N* sulfuric acid, 3.0 ml. of potassium iodide, and 4.0 ml. of starch, diluted to 50.0 ml., is used in the reference cell of the spectrophotometer. The solution is kept out of direct sunlight and after 30 to 45 minutes its percentage transmittance is measured. The micrograms of iodine are read from a standard curve and the concentration of iodine in the sample is calculated.

Table I. Recovery and Reproducibility Tests of Method

Material	Known Iodine Content	Iodine by Chromium Trioxide Method		Recovery or Deviation	
	P.p.m.	*P.p.m.*[a]	*P.p.m.*[b]	%[a]	%[b]
Standard iodine solution	5.00	5.00	4.98	100.0	99.6
Kelp[b]	1270.0	1268.0	1268.0	99.8	99.8
Wheat	.....	2.32	2.32	0.0	..
Bluegrass I	.....	4.00	4.10	2.5	..
Bluegrass II	.....	1.12	1.12	0.0	..
Fertilizer I	.....	105.0	105.0	0.0	..
Fertilizer II	.....	14.84	14.84	0.0	..
Fertilizer III	.....	4.90	4.90	0.0	..

[a] Analysis of 60 samples of oats showed average deviation 0.9% and maximum deviation of 2.5%.
[b] McHargue (*8*).

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Photometric Determination of Arsenic in Copper and Copper-Base Alloys

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A method is described for the determination of arsenic in arsenical brass, arsenical copper, and fire-refined copper. It has also been successfully applied to lead- and tin-base bearing metals and to open hearth iron. The arsenic is first separated from other constituents of the sample as metallic arsenic by digestion at 80° to 90° C. with hypophosphorous acid in a 50% hydrochloric acid solution. The precipitated arsenic is collected on a fritted-glass filtering funnel of fine porosity, oxidized to arsenic acid by solution in 0.02 *N* iodine, and finally converted to a heteropoly blue compound by treatment with ammonium molybdate and hydrazine sulfate. The transmittancy of the blue compound is measured preferably at 840 millimicrons, although satisfactory measurement may be made at lower wave lengths. The method is somewhat more rapid than conventional distillation-volumetric procedures, and it allows the use of smaller samples and gives a better recovery of arsenic.

THE usual distillation-titration procedures for determining arsenic in copper and copper-base alloys (*1*) have the disadvantages of being rather lengthy and not well adapted to handling samples in batch lots, requiring the use of relatively large samples when the amount of arsenic is small and, in the author's experience, of giving variable recoveries of arsenic, usually low.

In attempts to overcome some of these disadvantages Rodden (*11*) and Luke (*9*) have devised photometric procedures utilizing the "heteropoly blue" compound formed by heating arsenic acid with an acidified solution of ammonium molybdate and hydrazine sulfate. This reaction is very sensitive and thoroughly reliable, but before it can be applied the arsenic must generally be separated from the bulk of the sample. Both of the photometric methods so far described for metallurgical materials make use of a distillation procedure for this separation, and both suffer some of the disadvantages noted above.

Apart from distillation procedures, the only other separation of arsenic of general application is the precipitation of metallic arsenic, either by stannous chloride or by hypophosphorous acid (*2, 4-6, 8, 10, 12, 13*). Hypophosphorous acid is usually considered to be the more sensitive and generally satisfactory reagent (*12*).

The method described in this paper, utilizing this separation, was devised to provide an accurate and reasonably rapid uniform procedure for handling all types of arsenical copper and copper-base alloys in batch lots. In practice one operator can analyze from 20 to 25 samples per day with an accuracy better than that obtained by the standard distillation-titration procedure.

SPECIAL APPARATUS

Spectrophotometer suitable for operation in the range of 700 to 900 millimicrons, or a filter photometer equipped with a filter having a maximum transmittance in this range.

Büchner funnel with fritted disk, 15- or 30-ml. capacity, fine porosity.

Bell jar with open top and side tubulature, and of sufficient height to contain a 200- or 250-ml. volumetric flask. A highly satisfactory substitute for the conventional bell jar may be constructed from a 2-liter Pyrex suction flask by cutting a hole 10 cm. (4 inches) in diameter in the bottom and grinding to a glass plate (obtainable from the Macalaster Bicknel Co. of Connecticut, Inc., New Haven, Conn.).

REAGENTS

Standard Arsenic Solution (1 ml. = 0.10 mg. of arsenic). Dissolve 0.1320 gram of pure arsenic trioxide (As_2O_3) in 5 ml. of 5% sodium hydroxide using gentle heat. Dilute to 100 ml. with water and just acidify to litmus paper with dilute sulfuric acid (1 to 1). Dilute to 1 liter in a volumetric flask.

Hypophosphorous Acid (50%) **or Sodium Hypophosphite Crystal** ($NaH_2PO_2.H_2O$). Calcium hypophosphite is not suitable for use in the presence of sulfates.

Iodine (0.02 *N*). Dissolve 2.54 grams of iodine and 8 grams of potassium iodide in 25 ml. of water. When solution is complete, dilute to 1 liter. Store in a cool place in a dark-colored, glass-stoppered bottle.

Aerosol (10%).

Ammonium Molybdate (1% in 5 *N* sulfuric acid). Add 70 ml. of sulfuric acid to about 300 ml. of water, stirring during the addition. Dissolve 5 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in the warm acid mixture. When solution is complete, cool and dilute to 500 ml.

Hydrazine Sulfate (0.15%). Dissolve 0.30 gram of C.P. hydrazine sulfate, $N_2H_4.H_2SO_4$, in about 150 ml. of water and dilute to 200 ml.

PREPARATION OF CALIBRATION CURVE

The concentration range covered at 840 millimicrons using a cell depth of 1 cm. is from 0.01 to 0.075 mg. of arsenic per 25 ml. The following directions for preparing a calibration curve are based on the above conditions.

Transfer seven 1.00-gram portions of high-purity electrolytic copper containing less than 0.001% arsenic to 150-ml. beakers. Add from a microburet 1.0-, 2.0-, 3.0-, 4.0-, 5.0-, 6.0-, and 7.0-ml. portions of standard arsenic solution equivalent to 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, and 0.70 mg. of arsenic.

Dissolve the copper in 4 ml. of nitric acid, add 2.0 ml. of sulfuric acid and evaporate the solutions until the salts are gray in color and barely moist with sulfuric acid.

Allow the samples to cool and dissolve the salts in 25 ml. of water. Add 30 ml. of hydrochloric acid followed by 5 ml. of hypophosphorous acid (or 4 grams of sodium hypophosphite), stirring between additions. The solutions should be nearly colorless at this point.

Cover the solutions, heat to 80° to 90° C., and hold at this temperature for 10 to 20 minutes (or until no further darkening of the solutions is apparent) to complete precipitation of the arsenic. Then boil gently for 3 to 5 minutes to coagulate the finely divided precipitate.

Filter through a fritted-glass funnel of fine porosity, using moderate suction. Do not fill the funnel more than two thirds full, as the precipitate has a tendency to creep.

Wash the beaker three times with hot freshly boiled water, pouring the washings through the filter. Then wash the funnel three times. Discard the filtrate, which should be sparkling clear and very nearly colorless.

Place a 200-ml. volumetric flask inside the bell jar and insert the fritted-glass funnel containing the arsenic in the top of the bell jar so that the funnel stem reaches into the neck of the volumetric flask.

Rinse the beaker in which the precipitation was made with 10 ml. of iodine solution to which has been added 1 drop of Aerosol, and pour the iodine into the funnel containing the arsenic. Stir the solution in the funnel with a thin glass rod tipped with a rubber policeman and scrub the funnel sides (especially the upper portion) until the arsenic is dissolved.

Apply gentle suction to draw the iodine through the filter at the rate of about 1 drop per second and stir occasionally while draining.

Wash the beaker three times with hot water, pouring the washing through the filter, and follow with three washes on the funnel.

Remove the volumetric flask containing the iodine solution of the arsenic from the bell jar, dilute to the mark, and mix.

Transfer a 20-ml. aliquot to a 25-ml. volumetric flask. Add 2.5 ml. of ammonium molybdate solution and 1.0 ml. of hydrazine sulfate solution, mixing between additions. Heat the flask in a water bath at 90° to 100° C. for 10 minutes, cool to room temperature, dilute to the mark, and mix.

Transfer a portion of the solution to a suitable absorption cell and read transmittancy (or density) against distilled water, preferably at 840 millimicrons, though lower wave lengths down to 700 millimicrons may be used. Plot photometer readings against milligrams of arsenic on suitable coordinate paper. At 840 millimicrons the graph should be a straight line.

For photometers using test tubes for absorption cells a somewhat simpler procedure is available. In such a case, collect the iodine solution of the arsenic in a 250-ml. volumetric flask, dilute the solution to about 100 ml. wih water, and add 25 ml. of ammonium molybdate solution and 10 ml. of hydrazine sulfate solution directly to the flask. Dilute to the mark and mix. Fill a clean, dry, absorption tube to a reference mark and heat the tube in a water bath as described above; cool, dilute to the reference mark to replace water lost by evaporation, mix, and read the color value directly.

ANALYTICAL PROCEDURE

Arsenical Brass (range 0.02 to 0.10% arsenic). Transfer a 1.00-gram sample to a 150-ml. beaker, dissolve in 8 ml. of dilute nitric acid (1 to 1), add 2 ml. of sulfuric acid, and proceed as in the preparation of the calibration curve. Should the arsenic content prove to be beyond the range of the calibration curve, take a 10-ml. aliquot of the iodine solution of the arsenic, dilute to 20 ml., and proceed as usual.

Arsenical Copper (range 0.15 to 0.50% arsenic). Transfer a 0.20-gram sample to a 150-ml. beaker, dissolve in 3 ml. of dilute nitric acid (1 to 1), add 1 ml. of sulfuric acid, and proceed as for Arsenical Brass.

Fire-Refined Copper (range 0.002 to 0.012% arsenic). Transfer a 5.00-gram sample to a 400-ml. beaker, dissolve in 30 to 40 ml. of dilute nitric acid (1 to 1), add 8 ml. of sulfuric acid, and evaporate to near dryness. Dissolve salts in 60 ml. of water, add 75 ml. of hydrochloric acid and 15 ml. of hypophosphorous acid (or 12 grams of sodium hypophosphite), and digest the solution at 80° to 90° C. for 30 minutes or longer. Then boil for 5 minutes, filter, dissolve the arsenic, and develop the color as usual.

DISCUSSION

Under the conditions outlined above the only elements encountered that precipitate with the arsenic are selenium and tellurium, and these do no harm in moderate amounts. Evans (*6*) states that tin and antimony interfere and that alloys containing large amounts of one or both require special treatment; however, the author's experience, as well as that of others (*2*, *8*), does not support this contention.

Insoluble material (such as silica) must be filtered off prior to precipitation of the arsenic; however, moderate amounts of lead sulfate will go into solution when the hydrochloric acid is added (*10*).

There appears to be some controversy about the proper washing of the filtered arsenic. Evans (*6*) states that the precipitate must first be thoroughly washed with a 2% solution of sodium hypophosphite in 1 to 3 hydrochloric acid and finally with a 5% ammonium chloride solution. Sloviter (*12*) states that simply washing with hot water, freshly boiled to remove oxygen, is sufficient. The author's experience has been that hot water washing is sufficient, except in handling 5 gram samples, when a preliminary wash with 1 to 3 hydrochloric acid containing about 1% hypophosphorous acid is desirable to prevent the possible precipitation of cuprous chloride. It is essential, in any case, that the washing be carefully done, because any phosphorus compounds contaminating the precipitate or the filter interfere in the subsequent color development.

The author has found 0.02 *N* iodine to be a reliable solvent for small amounts of arsenic, provided the iodine is not drawn through the filter too fast and the solution is thoroughly stirred before suction is applied and occasionally during draining. The excess iodine is bleached by reduction during the heating with hydrazine sulfate.

The heteropoly blue reaction, using hydrazine sulfate as the reducing agent, has been treated in a recent review (*3*). Several elements, notably phosphorus, may interfere, but none will be present in the iodine solution of the arsenic. Some excess of hydrazine sulfate over that usually prescribed is necessary for reduction of the excess iodine. There seems to be no necessity for the common practice of mixing the acidified molybdate with the hydrazine sulfate prior to addition to the sample. The mixed solution is unstable, whereas the separate solutions will keep for a considerable length of time.

The recovery of arsenic is not quantitative, averaging about

Table I. Recovery of Arsenic Precipitated from 1.00 Gram of Copper

(Evelyn photoelectric colorimeter)

	First Series			Second Series		
Arsenic Added, Mg.	Transmittance, No. 720 filter	Arsenic recovered[a], mg.	Recovery, %	Transmittance, No. 720 filter	Arsenic recovered[a], mg.	Recovery, %
0.10	73.25	0.100	100.00	73.75	0.098	98.00
0.20	55.75	0.188	94.00	55.75+	0.187	93.50
0.30	41.25	0.287	95.67	41.75	0.283	94.33
0.40	31.00	0.380	95.00	30.75+	0.381	95.25
0.50	24.00	0.468	93.60	23.75	0.472	94.40
0.60	17.75+	0.575	95.83	17.25+	0.585	97.50
0.70	14.25	0.658	94.00	13.75	0.670	95.71
		Av.	95.44			95.53

[a] Values obtained from calibration curve prepared by direct color development of standard arsenic solutions.

Table II. Results of Application of Method to Analysis of Commercial and Bureau of Standards Samples

(Beckman spectrophotometer, Model DU; 1-cm. cells)

Material	Sample Weight, Gram	Density, 840 Mμ	Equivalent Arsenic, Mg.	Arsenic Photometric Method, %	Arsenic Conventional Methods, %
Arsenical admiralty metal[a]	1.0022	0.561	0.0442	0.044	0.037[b]
	1.0005	0.554	0.0437	0.044	
	1.0024	0.573	0.0452	0.045	
Arsenical aluminum brass[c]	1.0002	0.857	0.0673	0.067	0.063[b]
	1.0040	0.874	0.0686	0.068	
	1.0005	0.821	0.0645	0.064	
Phosphorized arsenical copper[d]	0.2005	0.562	0.0442	0.220	0.195[e]
	0.2000	0.555	0.0438	0.219	0.195
	0.2032	0.553	0.0440	0.217	
Fire-refined copper[f]	5.0018	0.270	0.0215	0.0043	0.0049[g]
	5.0038	0.230	0.0185	0.0037	
	5.0054	0.258	0.0207	0.0041	
Phosphor bronze bearing metal[h]	1.0006	0.408	0.0323	0.032	Certificate value
	1.0011	0.395	0.0313	0.031	0.027[i]
	1.0019	0.374	0.0296	0.030	Range 0.025–0.030
Lead-base bearing metal[j]	1.0011	0.828	0.0652	0.065	Certificate value
	1.0009	0.846	0.0665	0.066	0.069[i]
	1.0029	0.824	0.0649	0.065	Range 0.058–0.075
Tin-base bearing metal[k]	1.0016	0.697	0.0550	0.055	Certificate value
	1.0009	0.690	0.0543	0.054	0.05[i]
	1.0012	0.682	0.0537	0.054	Range 0.02–0.07
Open hearth iron[l]	1.0017	0.173	0.0140	0.014	Certificate value
	1.0028	0.176	0.0142	0.014	0.012[i]
	1.0031	0.172	0.0137	0.014	Range 0.010–0.013

[a] Cu 70.00, Zn 28.95, Sn 1.00, As 0.05 (nominal composition).
[b] Determination by A.S.T.M. method (*1*) using 5-gram sample.
[c] Cu 76.00, Zn 21.95, Al 2.00, As 0.05 (nominal composition).
[d] Cu 99.767, P 0.022 (actual analysis).
[e] Determination (*1*) using 2-gram sample.
[f] Cu 99.899, Ag 0.0095, O 0.0422, Ni 0.0157, other impurities less than 0.005 each (actual analysis).
[g] Determination (*1*) using 100-gram sample. Value may be slightly high due to possible contamination of distillate with selenium.
[h] Bureau of Standards standard sample 63a. Cu 78.48, Pb 8.92, Sn 9.76, Zn 0.61, P 0.58, Fe 0.52, Sb 0.49, Ni 0.32, S 0.11 (certificate values).
[i] See certificate for methods used.
[j] Bureau of Standards standard sample 53a. Pb 79.37, Sb 10.29, Sn 10.23, Bi 0.054, Fe 0.006, Cu 0.002, Ni 0.006, Ag 0.006 (certificate values).
[k] Bureau of Standards standard sample 54. Sn 88.24, Sb 7.33, Cu 3.75, Pb 0.56, Fe 0.06, Bi 0.05 (certificate values).
[l] Bureau of Standard standard sample 55a. Total of 16 impurities 0.17, balance Fe (certificate values).

95% for a range of 0.10 to 0.70 mg. as shown in Table I. As the loss is small and remains constant and the method is standardized empirically, this fact is of little practical consequence.

Various means of improving recovery have been tried, such as increasing the amount of hypophosphorous acid, varying the amounts of hydrochloric acid, heating under a reflux condenser, and adding a trace of mercuric chloride to catalyze the reduction (*7, 10*); however, none of these expedients has resulted in an increased recovery.

APPLICATION OF METHOD TO COMMERCIAL AND BUREAU OF STANDARDS SAMPLES

Table II lists results obtained for several common arsenical copper-base alloys as well as for a lead-base and a tin-base alloy and for a sample of open hearth iron. In general, results obtained by the method described in this paper are slightly higher than those obtained by methods involving a distillation separation. This supports the author's contention as well as that of Evans (*6*), that distillation recoveries of arsenic are likely to be somewhat low.

Although the method described in this paper was developed primarily for application to copper and copper-base alloys, the results for the white metal alloys and the open hearth iron show that it is of rather general application.

The lead- and tin-base alloys were dissolved in 8 ml. of aqua regia, evaporated to dryness on a steam plate, treated with 5 ml. of hydrochloric acid, and again evaporated to dryness, and the analysis was completed as usual. Cuprous chloride (0.5 gram) was added to all samples that did not contain a substantial amount of copper, as copper salts apparently catalyze the reduction to metallic arsenic (*6*). The lead-base sample had to be kept hot during filtration to prevent crystallization of lead chloride. Further details on the analysis of white metal and ferrous alloys are given by Anderson (*2*) and Evans (*6*).

The successful results for the sample of open hearth iron indicate that extremely small amounts of arsenic in certain materials (such as electrolytic copper) may be gathered from a 25-gram or larger sample by coprecipitation with ferric hydroxide and determined in the presence of the iron.

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Measurement of Carbon 14

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A procedure for the measurement of radioactivity from C^{14} is described. The samples are converted to barium carbonate and the activity is measured with a modified Lauritsen electroscope.

A NUMBER of sensitive and accurate assay methods for C^{14} involve Geiger-Müller counters (*1–4, 8–11, 13, 14*) or electrometers (*4–6, 12*) and employ carbon dioxide or barium carbonate as the sample material. Excellent summaries of the properties and problems associated with the measurement of C^{14} have been provided by Kamen (*7*) and by Reid, Weil, and Dunning (*10*).

Most of the published procedures for assay of C^{14} are primarily intended for biological research where dilution factors are large and high sensitivity is required to obviate prohibitive activity levels. For chemical work the dilution factors are usually small, and it is often possible to sacrifice sensitivity for simplicity of apparatus and convenience of procedure.

The method for the determination of C^{14} in organic compounds described in this paper makes use of a combustion train to convert the organic materials to carbon dioxide. The carbon dioxide is precipitated as barium carbonate and subjected to radioactive analysis with a Lauritsen electroscope as modified by Henriques, Kistiakowsky, Margnetti, and Schneider (*4*) and by Seligman (*12*). To eliminate necessity for determining the thickness of the precipitates, the original sample weights were chosen so as to give sufficient barium carbonate for samples of thickness greater than the range of the C^{14} β-particles in barium carbonate (*10*).

EXPERIMENTAL

The apparatus for the precipitation of the radioactive carbon as barium carbonate is shown in Figure 1. The sintered-glass filters were prepared by the method of Henriques, Kistiakowsky, Margnetti, and Schneider (*4*). The filter paper was S and S Blue Ribbon (Schleicher and Schuell Co., New York, N. Y.), cut into 2.6-cm. circles. The Lauritsen electroscope (Fred Henson Co., Pasadena, Calif.) was modified so that the samples could be placed within the electroscope chamber directly below the quartz fiber (*4, 12*). The air in the electroscope chamber was kept dry by a tray of anhydrous magnesium perchlorate covered with lens paper. The combustion train was of the conventional semimicro design.

PROCEDURE

The precipitation apparatus was flushed thoroughly with carbon dioxide–free oxygen or nitrogen and 4 ml. of 0.5 *N* carbonate-free (*3*) sodium hydroxide solution were run into the absorption tube, which was connected to the combustion train. The weight of the sample of organic material was determined by the area of the barium carbonate filter and the range (17 to 19 mg. per sq. cm.) of the C^{14} β-particles in solid barium carbonate. In the authors' work most of the precipitates had an area of 2.9 sq. cm. and a sample large enough to give 50 to 55 mg. of barium carbonate was required. After the combustion was complete, the flow of gas was continued for 20 minutes to make sure that all the carbon dioxide was swept into the absorber. Four milliliters of a

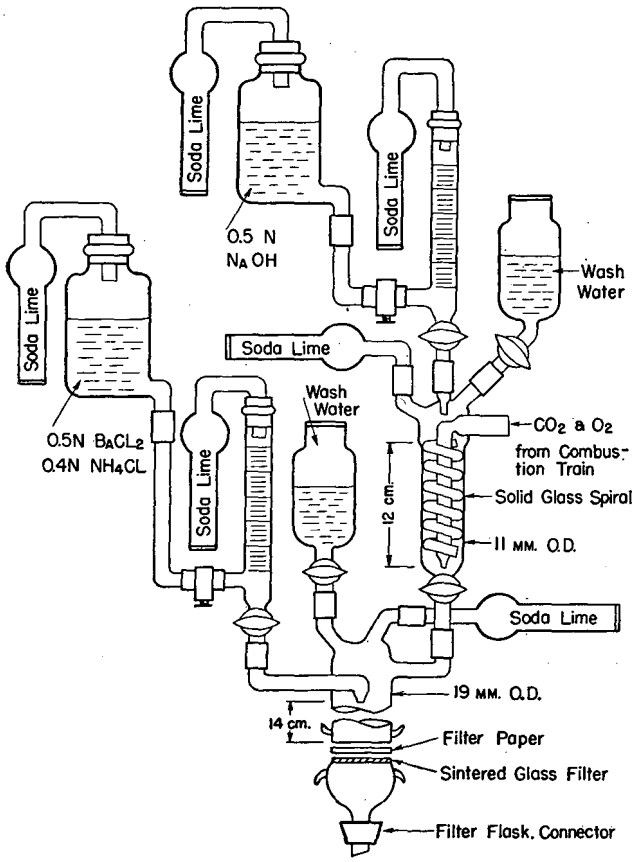

Figure 1. Apparatus for Precipitation of Radioactive Carbon

solution of 0.5 *N* barium chloride and 0.4 *N* ammonium chloride (*3*) were placed in the precipitation chamber with the suction turned off and the sodium hydroxide–carbonate solution was run in. The gas absorption spiral and tube were washed with three 5-ml. portions of boiling distilled water and the washings were added to the precipitation chamber. The precipitate was then filtered, using suction, and washed with five 5-ml. portions of boiling distilled water. With the suction on, the springs holding the filter together were removed, the precipitation chamber was raised, and the filter paper was removed from the sintered glass disk and placed in the brass holder (Figure 2). The sample was dried under an infrared lamp at a distance of 15 cm. (6 inches) for 10 minutes and then allowed to cool over anhydrous magnesium perchlorate in a desiccator. The precipitates were even, and if handled and dried carefully showed little tendency to develop serious cracks.

The sensitivity of the electroscope was checked with a uranium oxide source, after taking background measurements, just before the sample and holder were placed within the electroscope chamber. The electroscope used gave a fairly linear sensitivity curve from 10 to 40 scale divisions, and all measurements were made over this part of the scale. Reproducible activity readings were obtained only after the sample had been in the electroscope for 5 minutes. Thereafter, the time required for the fiber to travel from 10 to 40 on the scale was recorded.

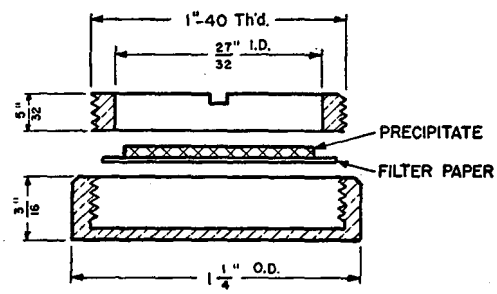

Figure 2. Sample Holder

Table I. Variation of Measured Activity with Thickness of Barium Carbonate Precipitate

Sample Weight, Mg.	$BaCO_3$, Mg./Sq. Cm.	Activity[a]
40.1	13.9	5.62 ± 0.06
44.7	15.4	6.56 ± 0.05
50.3	17.4	6.71 ± 0.06
51.3	17.7	6.79 ± 0.08
55.1	19.0	6.76 ± 0.04

[a] Electroscope scale divisions per minute corrected for background with standard deviations.

Table II. Analyses of Mixtures of Radioactive and Inactive Barium Carbonate

Standard Sample, Mg.	Total Sample, Weight, Mg	Activity[a]	Activity Calcd., %	Activity Found, %
55.1	55.1	6.75 ± 0.06	100.0	100.0
44.1	55.8	5.45 ± 0.05	79.0	80.7
43.2	55.3	4.96 ± 0.04	78.1	73.5
36.4	55.0	4.43 ± 0.08	66.2	65.6
32.5	56.0	3.95 ± 0.04	58.0	58.5
22.0	55.6	2.66 ± 0.04	39.6	39.4
16.4	55.0	1.97 ± 0.02	29.8	29.2
14.4	55.6	1.67 ± 0.02	25.9	24.7
5.6	55.9	0.674 ± 0.009	10.0	10.0

[a] Electroscope scale divisions per minute corrected for background with standard deviations.

Table I shows that the measured activity of a standard sample is independent of precipitate thickness with more than 17 mg. per sq. cm. of barium carbonate. These results were obtained by acidifying weighed samples of active barium carbonate and passing the resulting carbon dioxide into the precipitation apparatus with a stream of nitrogen.

The precision possible with this assay method is illustrated by the measured activities of samples obtained by diluting a standard barium carbonate sample with inactive material (Table II). The activity of the standard barium carbonate was 2046 counts per minute per milligram of barium carbonate as measured in a carbon dioxide gas counter (*2*).

The method clearly has adequate precision for virtually all chemical mechanism studies where sufficiently active material can be used.

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Precision Determination of Lead in High Grade Copper

Dithizone Color and Electrodeposition Gravimetric Methods

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The technique of the determination of lead in copper by the dithizone and electrodeposition methods is discussed in detail.

WHEN electrolytic copper is produced by the air oxidation of molten copper, it is important to determine its lead content (0 to 0.03%) because of the undesirable hardness attributed to the presence of small amounts of lead in highly refined copper metal. The most applicable chemical procedures are the lead dithizone photometric and lead dioxide electrodeposition methods. The techniques of these two methods are discussed in detail in this paper.

ELECTRODEPOSITION METHOD

Acidity. When lead dioxide is to be deposited from a copper nitrate solution of 200-ml. volume, the nitric acid content should be (roughly) proportional to the amount of lead present:

0.15 gram of lead, 15% of nitric acid (by volume)
0.05 gram of lead, 5 to 7% of nitric acid
0.002 gram of lead, 1 to 2% of nitric acid plus ammonium ion

These figures indicate the desired range of acidity, which must be kept in mind when preparing the electrolyte.

Ammonium and Nitrate Ions. Ammonium nitrate is an essential reagent in the electrodeposition of small amounts of lead dioxide. The electrolyte should contain a minimum of 5 grams of ammonium ion per 100 ml.; electrolytes containing less than this amount give poorer plates than those of higher ammonium contents. Ammonium nitrate increases the conductivity of the electrolyte (decreases the resistance), which controls the voltage drop across the electrodes.

Calculated in terms of molar concentrations, ammonium nitrate has the highest concentration of the compounds present. It is then proper to regard the electrolyte as an ammonium nitrate solution, acidified with nitric acid and containing copper and lead ions. In this procedure the molar concentrations of the constituents are: ammonium nitrate $-0.8\ M$, nitric acid $-0.1\ M$, and copper nitrate $-0.08\ M$.

Stirring. Mechanical stirring, either rotating anode or moving glass rod, radiates from the center of the solution and is more active at the inner than the outer electrode surface. As lead peroxide can often be shaken off the "rotating anode," it is common practice to reverse the electrical connections. In this way the lead peroxide plates on the outer, or stationary electrode, unaffected by mechanical stirring.

Absence of Lower Oxides of Nitrogen. When copper and copper-base alloys are dissolved in nitric acid, lower oxides of nitrogen are formed. These are reducing agents and would react chemically to dissolve the lead dioxide from the anode, but they may be expelled from solution by boiling. Many analysts insert glass beads or stirring rods into the solution, as aids in boiling out the gaseous oxides. In extreme cases, a small amount of sulfamic acid (*7*) may be added before electrodeposition is started.

Temperature of Electrolyte. The earlier investigators (Fischer, Smith, and Sand, *2*) plated out lead dioxide from hot nitric acid solutions, but general plating practice is to use solutions at room temperature.

Other Impurities. Many elements interfere with the accurate determination of lead as electrolytic lead dioxide. Mercury, selenium, phosphorus, and arsenic (*2*) retard or prevent complete deposition as dioxide, while bismuth, silver, thallium, manganese, and copper tend toward high results. However, in the processing of the molten copper, the first five elements would be volatilized or removed with the slag.

Bismuth, silver, thallium, and manganese may or may not plate out with lead dioxide on the anode, and therein lies the greatest error in the electrodeposition of lead dioxide from copper solutions. In the case of refined copper, tests indicate that bismuth and manganese have been removed in the manufacture, and that thallium was not present in the original scrap copper. Copper has been detected in the lead deposit, but in unweighable quantities. Hence, the only important element that interferes with the precision determination of lead as dioxide is silver. The correction may amount to several thousandths of 1%.

The presence of silver in refined copper metal may be detected by direct spot test on the metal, using *p*-dimethylaminobenzalrhodanine (*6*). Tin, if present, is oxidized to the insoluble tin dioxide, and may be removed by filtration.

Copper Ion. In low nitric acid concentration, lead tends to plate out on the cathode, if copper is totally absent (*2*). Thus, in this case the copper ion is an aid in the proper deposition of lead dioxide.

Electrical Conditions. Each ion has its own individual electroplating voltage, just as individual as the melting point of the metal. For example, from a solution of silver nitrate and copper nitrate all the silver can be electroplated at 1.2 volts while the copper will remain in solution until the voltage reaches 2.2 volts. Lead could be deposited as lead dioxide at 0.9 volt (*9*). However, an industrial laboratory is not equipped for such work. In practice, therefore, an electrical source of 6 to 12 volts is applied, with a current of 1 to 2 amperes. The voltage drop across the anode and cathode will be 2.2 volts, indicating that copper is being plated out. At the same time, lead deposits as lead dioxide on the anode. The laboratory conditions, then, are a direct current source which will be 6 or more volts and about 2 amperes. Plating time is usually 15 to 30 minutes. Theoretically, 0.02% lead should be plated out in less than 2 minutes.

Reagents. Nitric acid, specific gravity 1.42, C.P. reagent, has not been reported as containing even traces of lead. Ammonium hydroxide, specific gravity 0.9, C.P. reagent, must be tested for lead on a blank run. In cases of slight contamination, a constant blank must be subtracted—for example, 0.1 mg. per 20 ml. of ammonium hydroxide.

Apparatus. As a precaution, Pyrex beakers are boiled with nitric acid if contamination is suspected. Platinum gauze electrodes may be smooth or sand-blasted. The size of electrodes depends on availability. Electrodes containing 100 sq. cm. of surface area are "standard."

Procedure. PREPARATION OF SAMPLE. For rapid control tests, copper shot, made by dropping copper melt into cold water, is used. If the shot appears dirty, it should be cleaned with hydrochloric acid, rinsed with water, and dried. The final tests are usually made on drillings from the copper bars.

Weigh a 10.00 (±0.05) gram sample, and transfer to a 300-ml. tall-form beaker. Partially cover the beaker with a watch glass, add 50 ml. of water, and then add 50 ml. of nitric acid (specific

gravity 1.4) in small portions, so that the reaction does not become too rapid. Insert a stirring rod, slide back the watch glass part way, and heat to, and maintain at, the simmering point for about 20 minutes. This should expell all nitrogen oxides. Remove the beaker from the heat, cool in running water to room temperature, and note whether the odor of nitrogen peroxide is completely absent. (If the odor of nitrogen peroxide is detected, the solution must be reheated for 5 minutes.)

Dilute the solution to about 100 ml. with water. Slowly, and with stirring, add ammonium hydroxide until the solution is deep blue. A minimum of 20 ml. of ammonium hydroxide must be used. Again cool the solution in running water. Stir, and drop in nitric acid (specific gravity 1.4) until the deep blue color fades, then more carefully add nitric acid until the precipitate just dissolves. Follow this with a measured excess of not more than 5 ml. of nitric acid. Cool the solution to room temperature.

Clean a set of platinum electrodes in nitric acid, wash in distilled water, and ignite above a Fisher burner. Set up the electrodes, making the outer electrode the anode. Use an electrical source of 6 to 12 volts and 1 to 2 amperes and electroplate with mechanical stirring for 15 to 20 minutes. At the end of the plating time, stop the stirring, slowly lower the electrolytic beaker, and at the same time wash down the electrodes with distilled water. Turn off the current, and wash the anode in distilled water. Drain off excess water by resting the electrode on a towel, then dry at 110° C. for 20 minutes.

Remove the electrode from the oven and let stand near the balance for at least 5 minutes. Weigh the electrode as platinum plus lead dioxide. Strip the electrode in nitric acid (plus hydrogen peroxide), wash the electrode with distilled water, and then ignite the platinum over the Fisher burner. Cool the electrode and set it by the balance for 5 minutes. Weigh as platinum. Subtract this second weight from the original weight to obtain lead dioxide.

CALCULATION.

$$\% \text{ Pb} = \frac{\text{grams of } PbO_2 \times 0.866 \times 100\%}{\text{grams of sample}}$$

BLANKS. It is necessary to run a blank on each new batch of reagents. The nitric acid has been found to be lead-free, but samples of ammonium hydroxide show weighable quantities of lead. If cupric nitrate trihydrate is available, dissolve 40 grams in 100 ml. of water and 10 ml. of nitric acid and proceed as for lead in copper. If no lead is found, the copper nitrate may henceforth be used as blank. If the ammonium hydroxide is of uncertain purity, it is necessary only to redistill about 50 ml. and make a test run.

DITHIZONE COLORIMETRIC METHOD

Chemistry of Method (*1, 3, 4, 10, 11*). The sample is dissolved in nitric acid to form copper nitrate, lead nitrate, silver nitrate, etc. The oxides of nitrogen are boiled out, as they would react with the dithizone reagent. Tartaric acid and ammonium hydroxide are added with the formation of the blue copper ammonia ion. Tartaric acid aids in the smooth conversion of the blue cupric ion to the colorless cuprous cyanide ion, $Cu(CN)_2$ or $Cu(CN)_3$, depending on the amount of sodium cyanide present. Silver, iron, and nickel react in a similar manner, but lead remains as a tartrate. Potassium hydroxide is now added to raise the pH above 9 (*10*). A pH meter or thymolphthalein indicator may be used. The pH must be greater than 9 but less than 11 in order to extract the lead completely as dithizonate.

The solution is transferred to a separatory funnel and mixed with dithizone reagent. The aqueous and nonaqueous layers are separated, and the experienced analyst immediately decides on the next step. A red extract indicates that appreciable amounts of lead are present, a green color that lead is low, and intermediate shades show intermediate percentages. The extract, or extracts, are combined, and "washed" to remove excess green reagent; the aqueous wash solution is also given a nonaqueous extraction, as a precaution. The extracts are made up to volume with solvent, and the color intensity is determined photometrically, or by comparison with other standards. The color is stable for 8 hours. Maximum absorption occurs at 520 mμ.

This is the "one-color" system.

Adaptability of Procedure to High Grade Copper. As the material is 99.9% copper, the estimated impurities must necessarily be low. Thus, lead, if present to the extent of 0.01%, is to be considered a major constituent compared to any other foreign element present. In dithizone chemistry this is an important point, for extractions by immiscible solvents are based on partition coefficients, or per cent extracted (*10*).

If lead and element *E* (cadmium at pH 9.8) are present in equally small amounts, the lead dithizonate is extracted 100% and element *E* is extracted only 1%; but should *E* be present in 10 times the amount of lead, the lead is extracted 100% and *E* is extracted 10%, roughly in proportion to lead. Arithmetically, if lead were present to the extent of 0.01%, in the first example the color extract would appear to be 0.0101% lead (0.010%), and in the second case the color extract would appear to be 0.011% lead (based on equal color intensities per unit of weight for lead and element *E*). Element *E* by coextraction interferes in the second, but not in the first, case.

At this point the extraction of the elements from the test solution is completed, and the washing of the dithizone extract by an aqueous solution at selected pH is performed for the double purpose of removing excess dithizone reagent and element *E*. If the pH be such that lead is 100% extracted and *E* is only 5% extracted, in each case all the lead will remain in the organic layer (100%) along with 5% of the amount of element *E*, which is a 1 to 20 partition ratio. Arithmetically, in the first example the result will be 0.010% lead, and in the second case 0.01005% (or 0.010%) lead.

This demonstrates the advantages of applying aqueous backwashings to the nonaqueous extract. Wichmann's (*10*) graphs are useful in selecting the optimum working technique.

The copper readily lends itself to the dithizone reaction because the cuprous cyanide complex does not react with dithizone. When a 0.5-gram sample is used, the sodium cuprocyanide salt is without effect on the dithizone reagent or the lead dithizonate compound.

On these two points, that the base metal of high grade copper is unaffected by dithizone, and that the impurities are too small in amount to interfere, lies the usefulness of this procedure.

Errors Caused by Reagents. The trouble with the lead dithizone method is not any of the modified techniques offered, but rather the reagents.

Reagents introduce two types of errors. The first is high positive blanks caused by the presence of lead in the reagents. The purification of reagents (for lead) has been thoroughly discussed (*1, 4, 11*). The second type of error, low lead results caused by poor reagents, has been completely neglected.

Consider the test for 0.02% sulfide ion in sodium cyanide (*5*). If a silver salt is added to sodium cyanide solution containing sodium sulfide, a brown coloration of insoluble silver sulfide is formed. Lead would give lead sulfide; and lead sulfide is insoluble in dilute acids, alkalies, tartrates, and cyanides and does not react with a chloroform solution of dithizone. Applying this fact to the analytical procedure, if the reagents contained sulfide ion, the results for lead would be low, and the blanks would be called negative. Fortuitously, silver may be present in the copper in greater amounts than the lead, and react with most of the sulfide ion.

Tartaric acid and carbon tetrachloride were chosen, instead of citric acid and chloroform, because the citric acid samples contained lead, and carbon tetrachloride separates more clearly from a pH 10 solution (*10*).

Interfering Elements. When present in 99.9% copper, tin, iron, nickel, zinc, phosphorus, and silver do not interfere. Tin is removed by filtration; iron is converted to ferricyanide and, if present in quantities of 10 mg. or more, would interfere by oxidizing the dithizone; nickel and zinc are converted to complex cyanides which do not react; phosphorus would give a precipitate of phosphate only in high concentrations; and silver (2 mg.) is inactive at the operating pH (*10*).

Amount of Lead Present. The purpose of refining the copper is to obtain a low lead content. As accurate analysis of 0.05% lead is of only cursory interest, the technique is aimed for a lead content of 0.02% or less. For this reason the analyst need plan for only a limited range of lead concentration. The size of sample, portions of reagents, numbers of extractions, and absorption curve are definite, and precision is obtained because of these limitations. This advantage is apparent, for any sample within the range may be handled with facility, and any sample whose lead content is too high may be discarded as unsatisfactory after the third dithizone extraction.

Reagents. DITHIZONE SOLUTION. Dissolve 0.010 gram of dithizone in 500 ml. of C.P. carbon tetrachloride. Keep in a cool, dark place. When a red tint appears in the bottom of the glass container, discard the reagent. The reagent will keep for 5 days in an electric refrigerator.

NITRIC ACID, 1%. One ml. of nitric acid in 100 ml. of water.

TARTARIC ACID SOLUTION. Dissolve 500 grams in warm water and dilute to 1 liter with water.

AMMONIA-CYANIDE REAGENT. Dissolve 10 grams of sodium cyanide in 200 ml. of water and 75 ml. of ammonium hydroxide (specific gravity 0.9), mix, and dilute to 500 ml. with water. Discard after 30 days.

THYMOLPHTHALEIN INDICATOR. Dissolve 0.04 gram in 100 ml. of ethyl alcohol. The indicator is blue, above pH 9.

POTASSIUM HYDROXIDE SOLUTION. Dissolve 50 grams in water and dilute to 100 ml. with water. Reagent sodium hydroxide was found to contain lead.

SODIUM CYANIDE SOLUTION. Dissolve 10 grams in water and dilute to 100 ml. with water.

STANDARD LEAD SOLUTION. Weigh 0.320 gram of lead nitrate (or 0.268 gram of lead chloride) and transfer to a 1-liter volumetric flask. Dilute to the mark with a 1% nitric acid solution, and mix well. Pipet 100 ml. of this solution into a second 1-liter volumetric flask, and dilute to the mark with 1% nitric acid solution. Mix well. The first volumetric flask contains the stock reagent (1 ml. = 0.2 mg. of lead), and the second volumetric flask contains the standard lead solution (1 ml. = 0.02 mg. of lead).

COPPER NITRATE TRIHYDRATE, lead free, used for running blanks on the reagents.

Blank Runs. First, examine each reagent separately by diluting 25 ml. of solution with 75 ml. of water and passing in an active stream of hydrogen sulfide. The solutions should become light yellow, and no dark brown color should be detected.

Run through the procedure, once with copper nitrate and once with no metal, up to the point where the extraction is to be made. Pass in hydrogen sulfide to detect the presence of lead, silver, or thallium.

Make complete runs, according to the procedure, one without metal and one containing 0.5 ml. of standard lead solution. The first should give a colorless or nearly colorless extract, but the second should be distinctly red.

Finally, make a sulfide test (*5*)—i.e., a blank run without metal to the point where sodium cyanide has been added. Add 1 ml. of a 1% silver nitrate solution. The solution should not turn brown.

In case any, or all, of the reagents show as much as 0.01 mg. of lead, they should be discarded rather than purified.

Colorimetric Procedure. PREPARATION OF SAMPLE. The remarks made under the "Electrodeposition Procedure" apply.

Weigh a 5.00-gram sample, transfer to a 300-ml. beaker, and dissolve in 25 ml. of water and 30 ml. of nitric acid. Cover the beaker with a watch-glass, insert a stirring rod, and boil for 2 to 3 minutes. Cool the sample to room temperature in running water. [If a small, white (not curdy) precipitate appears, it is tin oxide. A curdy precipitate indicates silver chloride. The precipitate will not interfere.]

Transfer the solution to a 250-ml. volumetric flask, dilute to the mark with water, and mix well. Pipet 25 ml. (0.50 gram) back into the original 300-ml. beaker and add 5 ml. of tartaric acid solution and 4 ml. of ammonium hydroxide (specific gravity 0.9). (Should the solution turn deep blue, decolorize it by adding several drops of tartaric acid.) At this point a precipitate may appear, but the supernatant liquid should be blue to green, not deep blue. Again, cool the solution to room temperature in running water. Carefully drop in potassium hydroxide solution (2 to 3 ml.) until the white precipitate which first forms (cupric hydroxide) redissolves and a clear blue solution is obtained (complex copper tartrate). Cool again. Slowly, and with stirring, add sodium cyanide solution (20 to 30 ml.) until the blue color changes to a light yellow or water-white solution. Add 4 drops of thymolphthalein indicator, and adjust the pH of the solution as follows:

If the solution is deep blue (alkaline), add tartaric acid until the solution is pale blue, then make the solution blue with ammonium hydroxide; if the solution is only pale blue, make it a deeper blue with ammonium hydroxide.

Transfer the solution to a 250-ml. separatory funnel, add 25 ml. of dithizone reagent, shake 30 seconds, then let settle for 2 minutes.

1. The lower (carbon tetrachloride) layer is a deep rose color (lead, 0.015% or more). Run the lower layer into separatory funnel 2, and reserve the extract. Add 25 ml. more of dithizone to funnel 1, shake 30 seconds, and let settle for 2 minutes. If the extract is again deep rose (lead is high), run the lower layer into funnel 2, and re-extract with 25 ml. of dithizone. (When the third extract is deep rose, there is too much lead present, and the samples may be discarded.) Continue as in 2 or 3.

2. The lower layer is light pink (lead less than 0.015%). Run the lower layer into funnel 2, and re-extract the upper layer with a mixture of 15 ml. of dithizone solution and 10 ml. of carbon tetrachloride, as before. Run the lower extract into funnel 2. Extract once more with only 15 ml. of carbon tetrachloride (no dithizone), and run into funnel 2.

3. The lower layer is an off-shade color of red and green, or green only. Run the lower layer into funnel 2, and extract the upper layer with only 25 ml. of carbon tetrachloride.

Discard the upper (aqueous layer) in funnel 1.

Prepare wash reagent as follows: Place 25 ml. of 1% nitric acid in a beaker, add 2 drops of thymolphthalein indicator, and add ammonia-cyanide reagent (about 5 ml.) until the solution is deep blue (pH 10). Pour this reagent into funnel 2, onto the red extracts. Shake 30 seconds and let stand for 2 minutes. Place funnel 2 above funnel 1, and run the lower (red) layer into funnel 1. Dry the outlet tips of the two funnels with rolled filter paper. Filter all but about 1 ml. of the red liquid through a dry filter paper into a dry 150-ml. beaker. Now extract the aqueous layer in the top funnel with 10 ml. of carbon tetrachloride, shaking and settling as usual. Carefully run the lower (carbon tetrachloride) layer from the top funnel to the bottom funnel, sealing off any of the water layer. Mix the contents of the lower funnel, then filter all but the last drops into the beaker, using the 10 ml. of carbon tetrachloride to wash the filter paper. Transfer the filtrate to a dry 100-ml. volumetric flask. Dilute to the mark with carbon tetrachloride. (In ordinary cases, the combined nonaqueous extracts do not exceed 75 ml.)

Determine the transmittance photometrically (520 mμ), using carbon tetrachloride as 100%, and obtain per cent of lead from the curve. Alternatives are to compare with standard lead solutions by eye or using a Duboscq, or compare with known color standards (*8*).

Preparation of Color Standards and Transmittance Curve. BLANK. Place 50 ml. of 1% nitric acid in funnel 1, and add 2 drops of thymolphthalein indicator and sufficient ammonia-cyanide reagent to color the solution blue. Extract with a mixture of 5 ml. of dithizone and 20 ml. of carbon tetrachloride, and complete the procedure beginning with 3 above, ending with a 100-ml. blank, which should be colorless. (This is not a reagent blank, only the blank used in preparing the standard transmittance curve. This is the solution for 100% setting.)

Prepare samples as above, using 1 and 3 ml. of standard lead solution, extracting with mixtures, according to 2 for the 1 ml. of lead standard solution. Make as many samples as desired using 4, 5, 6 ml., etc., of lead standard solution. Obtain photometric readings. Plot transmittance (ordinate) against lead (abscissa) on semilog graph paper.

General Notes. New standardization curves should be plotted every 6 months, as there may be changes in the light bulbs and filters. Each new lot of each reagent must be tested. It is suggested that selected reagents be reserved for this type of colorimetric work. It is a good plan to run an occasional blank with pure copper or pure copper nitrate, in order to check on possible reagent contamination; and a record check may be obtained by adding a known amount of standard lead solution to a blank run.

Table I. Determination of Lead in Refined Copper

Sample No.	Colorimetric, %	Electrodeposition, %
1	0.008	0.010
2	0.009	0.008, 0.009
3	0.009, 0.009	0.011, 0.011
4	0.009, 0.009	0.011
5	0.011	0.010, 0.011
6	0.012	0.010
7	0.014	0.009
8	0.016	0.016, 0.016
9	0.020	0.022
10	0.020	0.026
11	0.019, 0.020	0.026, 0.026
12	0.028	0.031, 0.031
13	0.028	0.032, 0.032
14	0.040, 0.042, 0.046	0.062, 0.062[a]
15	0.020[b]	0.018[b]
16	0.022[b]	0.022[b]
17	0.011[c]	

[a] Results by two analysts. Presence of silver (>0.1%) was suspected.
[b] 0.03% Sn present.
[c] Bureau of Standards sample 52a, 0.012% Pb. 5-gram sample used.

EXPERIMENTAL RESULTS

To illustrate the effect of acidity on the electrodeposition of lead, three 10-gram samples of copper (0.035% lead) were dissolved in 50 ml. of water and 40 ml. of nitric acid, then evaporated to dryness, cooled, and diluted with water. Then 10, 20, and 30 ml. of nitric acid were added to the respective beakers, and the solutions were boiled, cooled, and electrolyzed at 1 ampere for 30 minutes. The amounts of lead recovered were as follows:

10 ml. of nitric acid, 0.03% Pb
20 ml. of nitric acid, 0.02% Pb
30 ml. of nitric acid, 0.0% Pb

This amply illustrated the hindering effect of excess nitric acid.

A series of check samples was run by the colorimetric and electrodeposition methods (Table I). Polarographic examination of the impurities of the lead dioxide deposits is not within the scope of this paper (*1*).

The techniques described cannot be considered original, as these general methods have been in use for many years. The author invites comment on the discussions and takes this opportunity to thank associates for assistance rendered.

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Analysis of Sulfite Waste Liquor and Lignosulfonates

Determination of Neutralized Solids and Wet Oxidation for Determination of Total Sulfur and Cations

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A method is presented that allows a more complete determination of sulfite waste liquor components than the present total solids method. In the procedure described, neutralization of the sulfite waste liquor with caustic soda prior to drying prevents the loss of volatile organic acids and sulfur dioxide. A simple, rapid procedure for analyzing sulfite waste liquor and lignosulfonates involving oxidation of the organic matter by a nitric-perchloric acid mixture is described. This method may be used with either liquid or solid samples and it permits ready determination of all cations, silica, and total sulfur using the material from one digestion. The precision in the determination of sulfur is equal to that obtained in the Carius method.

SULFITE waste liquor is the spent cooking liquor that drains from the cellulose pulp made by digesting wood chips at elevated temperature and pressure with an aqueous solution of sulfurous acid and bisulfite. This waste liquor contains in soluble form the main part of the noncellulose components of wood. These components are mainly lignin, present as lignosulfonates, and hemicelluloses, present as partially or wholly hydrolyzed carbohydrate compounds. Because sulfite waste liquor organic matter usually comprises about 50% of the wood, it represents large amounts of organic matter which may give rise to objectionable pollution when passed into rivers and other waterways. Furthermore, this organic matter represents potential economic value.

For these reasons sulfite waste liquor disposal and utilization procedures are being studied with increasing interest. Such studies have been handicapped by lack of suitable analytical procedures. Partansky and Benson (*15*) have published procedures which have been adopted as TAPPI Standard Methods (*20*) and another set of standard methods has been adopted by the German Pulp and Paper Chemists and Engineers Association (*3*).

The procedures presented herewith have been used for a number of years. They have been found convenient and accurate for research as well as control in the field of sulfite waste liquor. One method is for the determination of "neutralized solids" in sulfite waste liquor. These neutralized solids are the solids obtained upon drying sulfite waste liquor which has been previously adjusted to a pH in the range from to 8.5 to 9.0 with sodium hydroxide. Another method is for the wet oxidation of either solid or liquid samples, whereby the organic matter is completely oxidized and the resulting solution can be used for determination of the inorganic sulfite waste liquor components such as total sulfur, silicon dioxide, ferric oxide, aluminum oxide, calcium oxide, magnesium oxide, and sodium oxide by known procedures.

DETERMINATION OF NEUTRALIZED SOLIDS FROM SULFITE WASTE LIQUOR

Sulfite waste liquor normally has a pH of from 1.5 to 3.0 and will therefore lose volatile acid components when evaporated to dryness. These acids are partly organic, such as acetic and formic, and partly sulfurous acid present in the original liquor as free sulfur dioxide, bisulfite, and "loosely combined" (aldehydic) sulfur dioxide. By neutralizing the sample with a known amount of standard sodium hydroxide solution to pH 8.5 to 9.0 prior to the evaporation and drying, the loss of the volatile acids is prevented. Sulfur analyses by wet oxidation show that no sulfur compounds present in the original sulfite waste liquor are lost by evaporation and drying with the neutralized solids procedure.

Analytical Procedure. Pipet 10 ml. of sulfite waste liquor into a 250-ml. beaker containing 100 to 125 ml. of distilled water.

Titrate with 0.2 *N* sodium hydroxide to a pH of 8.5 to 9.0 with the aid of a glass electrode pH meter. This titration is used to determine the sodium hydroxide required for neutralization.

Weigh a ground glass-stoppered weighing bottle to 0.0001 gram. Pipet another 10 ml. of sulfite waste liquor sample into the bottle and reweigh to 0.001 gram. Add the same amount of 0.2 *N* sodium hydroxide as was used in the above titration and mix well. Dry the neutralized sample in a constant temperature oven at 105° C., cool in a desiccator, and weigh to 0.0001 gram with cover on. For use in ordinary analysis 48 to 72 hours' drying is sufficient. After 96 hours there is virtually no loss in weight. From the dry weight subtract the tare weight of the dish plus the weight contributed by the sodium hydroxide added (milliliters of titration × normality of the sodium hydroxide solution × 0.022).

Calculations.

$$\% \text{ total solids} = \frac{\text{corrected weight of dried sample} \times 100}{\text{Weight of sulfite waste liquor}}$$

$$\text{Total solids, grams per liter} = \frac{\text{corrected weight of dried sample} \times \text{sp.gr. of sulfite waste liquor} \times 1000}{\text{weight of sulfite waste liquor}}$$

With ordinary sulfite waste liquor containing from 50 to 150 grams per liter of solids, this procedure will give results reproducible within 0.2 gram per liter or 0.02% solids.

Analytical Data. Table I illustrates the difference obtained when solids are determined according to the present standard method (*20*) by drying the sulfite waste liquor for 24 hours at 105° C. as compared with the neutralized solids procedure.

Table I. Sulfite Waste Liquor Solids

Total Solids *vs.* Neutralized Solids

Sample	Total Solids *G./l.*	Neutralized Solids *G./l.*
1	94.9	99.2
2	94.2	99.4
3	95.4	100.9
4	98.6	103.0
5	96.2	101.1

Discussion. The sulfite waste liquor samples used in the solids determinations given in Table I were of average free sulfur dioxide content. Under these conditions there is a difference of 4.0 to 5.5% in solids content between the two procedures. The true difference would be higher than shown, as the solids determined by the standard method do not come to constant weight in 24 hours.

WET OXIDATION FOR DETERMINATION OF TOTAL SULFUR AND CATIONS

This procedure consists in digesting the sulfite waste liquor sample with a mixture of nitric and perchloric acids until all organic matter is completely oxidized. Many analytical procedures, contributed mainly by Kahane (*4*) and Smith (*18*, *19*), are based on the use of perchloric acid for the rapid dissolving of samples and oxidation of organic matter. Wolesensky applied this principle for the determination of total sulfur in rubber (*23*) in a procedure based upon one of Kahane's (*5*). The authors' method is adapted from these published procedures.

The method as described will conveniently eliminate the organic matter and will enable the use of well-known standard methods for determination of silica and the cations present in sulfite waste liquor. The authors have found also that the oxidation quantitatively converts all sulfur compounds to sulfate, which is readily determined in a suitable aliquot of the digested sample. The digestion procedure therefore gives a convenient way for determining total sulfur in sulfite waste liquor.

The most generally accepted method for determining total sulfur, combined or mixed with organic matter, is the Carius method (*21*). This is limited to solid samples, is time-consuming, and requires special equipment and considerable skill in glass blowing. However, because the Carius method is recognized for its accuracy, it was employed as a standard reference for the analysis of total sulfur in a number of solid sulfite waste liquor and lignosulfonate samples which had been analyzed previously by the nitric-perchloric acid oxidation procedure described here.

The accuracy of the wet oxidation method has been demonstrated repeatedly when used for the analysis of aqueous solutions of sulfite waste liquor and lignosulfonates as well as of solid samples. This is a distinct advantage over procedures requiring a solid sample, as it eliminates the time required for drying and allows the use of a liquid sample, thereby simplifying precautions necessary to obtain a homogeneous sample.

Reagents and Apparatus for Wet Oxidation. C.P. nitric acid (70%), C.P. hydrochloric acid (37 to 38%), C.P. perchloric acid (60%), 100-ml. Kjeldahl flasks (Pyrex), and micro-Kjeldahl digestion shelf.

Analytical Procedure. If the sample is dried sulfite waste liquor, weigh 1 to 2 grams to the nearest 0.001 gram and introduce quantitatively into a 100-ml. Kjeldahl flask. If the sample is a liquid sulfite waste liquor, pipet out a 10.0-ml. sample into the Kjeldahl flask. In either case rinse down the neck of the flask with 10 to 15 ml. of distilled water. Next add 10 to 15 ml. of concentrated nitric acid and 5 ml. of perchloric acid. It is important in preventing explosions to have sufficient nitric acid present. Although the size of the sample used is the most important factor, the amount of nitric acid needed is dictated partially by the apparatus used and partially by the rate of digestion. If the digestion solution suddenly darkens after the main part of the excess nitric acid has boiled off, the digestion should be turned off immediately and more nitric acid added before the digestion is continued. This sudden darkening almost always indicates insufficient nitric acid. Until the worker is familiar with the apparatus and digestion procedure, maximum additions of the nitric acid are advisable.

Place the flask on a steam bath. When the oxidation reaction has subsided (usually after 15 to 20 minutes, as indicated by absence of the red-brown nitrogen dioxide fumes), transfer the flask to the digestion shelf and heat over the burner with moderate heat until oxidation is complete, as evidenced by a colorless solution and evolution of dense white perchloric acid fumes. Allow the solution to cool to room temperature, then add 5 ml. of concentrated hydrochloric acid and again heat on the shelf until the white fumes appear. Allow the solution to cool to room temperature, add 50 to 60 ml. of distilled water, and place on the steam bath to bring all salts into solution.

Normally sulfite waste liquor contains only small amounts of silica which usually are not determined. If a silica determination is desired, it is better to carry out the digestion in a beaker, thereby facilitating the quantitative transfer of the silica. In either case, filter off the silica on a filter paper and wash the precipitate with water. The silica is nongelatinous, owing to the dehydrating action of the perchloric acid, and can be determined either gravimetrically or colorimetrically (*12*, *22*).

From the filtrate precipitate the ferric hydroxide and aluminum hydroxide with ammonia by the established procedures (*7*) and heat on the steam bath until the precipitate flocks. Filter and wash the precipitate with a hot 1% ammonium chloride solution. Ignite the precipitate and weigh as ferric oxide plus aluminum oxide, or if individual analyses are desired, redissolve the precipitate in dilute hydrochloric acid and make the resulting solution to 100 ml. in a volumetric flask. Determine ferric oxide and aluminum oxide individually from aliquots of this solution by known methods (*8*).

Make the ammoniacal filtrate from the R_2O_3 precipitate to 250 ml. in a volumetric flask. Pipet out 100 ml. of this solution for determination of sulfur, adjust the pH of this aliquot with dilute hydrochloric acid to be just acid to methyl orange, and precipitate the sulfate with barium chloride. Filter, ignite, and weigh the precipitate according to the standard procedures (*9*) and calculate the sulfur.

Use the remaining 150 ml. of the ammoniacal solution for determination of calcium oxide as oxalate, which is filtered, redissolved, and titrated with 0.1 *N* potassium permanganate by the standard procedure (*10*). Use the filtrate from the calcium oxalate precipitate for determination of magnesium oxide. This is carried out conveniently by the bromometric 8-hydroxyquinoline method described in detail elsewhere (*11*, *16*).

Only certain types of sulfite waste liquor and lignosulfonates contain sodium ions. These can be determined conveniently after the digestion procedure described above.

It is best to take an aliquot for sodium determination from the filtrate after the removal of silica. Make this filtrate to 50 or 100 ml. in a volumetric flask and from this pipet 2 ml. or weigh approximately 2 grams into a 50-ml. beaker. Add 22 ml. of zinc–uranyl acetate reagent, stir, and let stand at least 30 minutes in a water bath at 20° ± 1° C. Filter and transfer the precipitate into a glass filtering crucible. Wash the beaker and precipitate in crucible with 15 to 20 ml. of anhydrous isopropyl alcohol in small portions. After drawing air through the crucible for 3 to 5 minutes, dry in an oven at a temperature of 105° C. to constant weight, which is usually established in 15 to 30 minutes.

This rapid method for sodium analysis has repeatedly given 99% of calculated values when samples with known sodium content have been used. For preparation of the reagent and additional information upon the procedures see, (*1*, *2*, *17*).

Table II. Determination of Total Sulfur

Sample No.	Type of Sample	Nitric-Perchloric Acid Method, %		Carius Method, %	
		1	2	1	2
1	Technical basic calcium lignosulfonate	4.61	4.61	4.70	4.71
		Av.	4.61		4.70
2	Technical sodium lignosulfonate	6.97	7.03	6.98	7.13
		Av.	7.00		7.06
3	Pure ammonium lignosulfonate	7.44	7.46	7.25	7.26
		Av.	7.45		7.26
4	Sulfite waste liquor solids	7.46	7.46	7.36	7.47
		Av.	7.46		7.42
5	Sulfite waste liquor solids	9.74	9.75	9.85	10.02
		Av.	9.74		9.94

Analytical Data. Dried and finely ground samples of sulfite waste liquor and lignosulfonates were used in the analysis for total sulfur, and the method described above was employed. The dried sulfite waste liquor samples were obtained by the neutralized solids procedure. The same samples were also used for total sulfur analysis by the Carius procedure. Analyses with both methods were carried out in duplicate for all samples. The results are given in Table II.

In Table III is given a comparison of the values obtained from the determination of total sulfur by means of the wet oxidation method, in one case using samples from neutralized solids and in the other case, liquid samples from the same liquor. In the former case, the dried solids were dissolved in water and then introduced into the Kjeldahl flask. In the latter, the analyses were made directly on a pipetted sample of 10.0 ml.

Table III. Total Sulfur-Sulfite Waste Liquor Solids *vs.* Liquid Sulfite Waste Liquor

Sample	Total Solids	Sulfur in Sulfite Waste Liquor Solids		Sulfur in Liquid Sulfite Waste Liquor
	G./l.	%	*G./l.*	*G./l.*
1	98.0	7.38	7.23	7.31
2	140.1	7.74	10.84	10.77
3	140.4	7.08	9.94	9.89
4	98.1	7.95	7.80	7.76

DISCUSSION

It is evident from the analytical data presented in Table II that the nitric-perchloric acid digestion procedure here described permits the determination of total sulfur in sulfite waste liquor and lignosulfonates with a precision equal to or better than that obtained with the Carius method. Table III demonstrates that the same results for total sulfur in sulfite waste liquor and lignosulfonates are obtained when the sulfite waste liquor is used in either liquid or neutralized solids form. It is thereby established that no part of the sulfur in sulfite waste liquor escapes oxidation to sulfate in the digestion oxidation.

The foregoing method for sulfur analysis was worked out for sulfite waste liquor and lignosulfonates. Aside from sulfonic sulfur, sulfite waste liquor contains sulfurous acid, bisulfite, sulfate, and often small amounts of thiosulfate and elemental sulfur. With the wet oxidation method, all these sulfur compounds are oxidized quantitatively to sulfate, simultaneously with complete destruction of the organic matter. It is likely that many organic sulfur compounds other than the lignosulfonates can be analyzed for total sulfur by this relatively simple method. For example, *S*-benzyl thiuronium chloride has been suggested (*14*) as a standard for checking the analysis of organic sulfur. A carefully purified preparation of this compound was analyzed for total sulfur by the wet oxidation method. The analysis showed 15.82 and 15.83% sulfur compared with 15.82% sulfur calculated for the pure compound.

In general, the wet oxidation procedure offers a simple, convenient, and rapid method for obtaining a solution of the inorganic constituents free from organic matter. Six digestions can be made simultaneously on the shelf; the oxidation occurs smoothly and requires a minimum of supervision by the analyst.

This digestion procedure has been used for years in the authors' laboratory and no untoward incident has been experienced. However, because perchloric acid when incorrectly applied or handled can produce dangerous explosions, it is recommended that the literature be consulted (*6*, *13*, *18*, *19*), particularly if its use is contemplated on organic material of a type different from that in sulfite waste liquor.

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Viscosity Blending Relationships of Heavy Petroleum Oils

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A total of 30 petroleum oil systems, using 17 different oils, was investigated. Each system was investigated at three or more compositions. The results were compared with viscosities predicted by a number of methods that have been proposed in the literature. The Wright method, a new way of using the A.S.T.M. chart for predicting blend viscosities, was found to give the best results in most cases. Many of the systems investigated were rather extreme, in that the component oils varied considerably in chemical nature. Two of these systems were found to exhibit definite viscosity minima. It was concluded that the use of viscosity-temperature characteristics of the component oils, as in the Wright method, is a definite improvement in viscosity prediction methods. However, sometimes differences in oils are not indicated by these relationships, but do affect blend viscosities to a considerable extent.

WHEN oils of different viscosities are mixed, the resulting blend usually has a lower viscosity than would be computed by assuming that viscosities are additive.

In 1887, Arrhenius (*3*) proposed a mixture rule in which log viscosity was taken to be additive. Later, Bingham (*5*) proposed reciprocal viscosities or fluidities, and in 1917 Kendall and Monroe (*11*) proposed cube root of viscosity as the additive function. Expressed as equations, these rules are:

$$\log\eta_{1,2} = x_{v_1} \log \eta_1 + x_{v_2} \log\eta_2 \quad \text{Arrhenius} \qquad (1)$$

$$1/\eta_{1,2} = x_{v_1}/\eta_1 + x_{v_2}/\eta_2 \quad \text{Bingham} \qquad (2)$$

$$\eta_{1,2} = x_1\eta_1^{1/3} + x_2\eta_2^{1/3} \quad \text{Kendall and Monroe} \qquad (3)$$

where η is viscosity in absolute units, x_v is volume fraction, and x is mole fraction. These three rules were all designed for "ideal" solutions and are generally not very accurate for use in predicting viscosities of petroleum oil blends, although (1) and (3) may have some application (*8*, *10*).

The several methods that have been proposed and used for predicting viscosities of petroleum oil blends are all empirical. Wilson (*16*) found that the viscosity of the blend depended not only on the amounts and viscosities of the oils being blended but also on the base or nature of the oil. His work led to three blending charts, one for blending oils of the same base, one for use when the higher viscosity oil is of paraffin base, and one for use when the higher viscosity oil is of naphthene base.

In 1932, the American Society for Testing Materials published a viscosity-temperature chart (*1*) which may also be used as a blending chart. This is the most widely used blending chart, but Wilson's charts are also used and have been reprinted in several standard books on petroleum refining (*4*, *13*).

At about the same time that the A.S.T.M. chart was first published, Cragoe (*6*) presented a comparable method. His viscosity function, L', is approximately additive for mixtures and so can be used in predicting the viscosities of blends.

In 1946, Wright (*17*) proposed a new method of using the A.S.T.M. chart for blending. The procedure is to plot the viscosity-temperature lines of the oils and then to "blend" by linear proportioning along the ln T axis. Thus, blending is done at "constant viscosity" rather than at constant temperature as in the conventional use of the chart. Figure 1 illustrates the use of Wright's method of predicting blend viscosities. This figure is included because Wright's paper has not yet appeared in any readily available publication.

In addition, several methods involving the use of correction factors have been proposed. Lederer (*12*) proposed the equation

$$\frac{x_{w1}}{x_{w1} + sx_{w2}} = \frac{\log \eta_{1,2} - \log \eta_2}{\log \eta_1 - \log \eta_2} \qquad (4)$$

where x_w is weight fraction and s is a constant, for any one system, which is evaluated by examining one blend experimentally.

Cragoe (*6*) in presenting his method also introduced an equation involving a correction factor:

$$L'_{1,2} = x_{w1} L'_1 + x_{w2}L'_2 + x_{w1}\, x_{w2}\, C \qquad (5)$$

where L' is Cragoe's viscosity function and C is a constant for any one system, again evaluated from the results of one blend. Such methods yield results that are usually of about the same order of accuracy as the experimental work.

EXPERIMENTAL WORK AND DISCUSSION OF RESULTS

In the experimental work, 30 binary petroleum oil systems using 17 different oils were investigated. Viscosities were determined at three or more compositions in each system. One purpose was to compare the different prediction methods as to applicability, and as a result many of the systems represent unusual or extreme cases. Modified Ostwald viscometers were used and viscosities were determined in accordance with A.S.T.M. standards (*2*). All blending was done volumetrically, and the over-all error of blending and viscosity determination is believed to be not more than 0.5%. Properties and the source of the 17 oils are given in Table I.

Systems of Large Viscosity Ratio. The first 13 systems exhibit a relatively large range of viscosity ratios (3 to 40) and

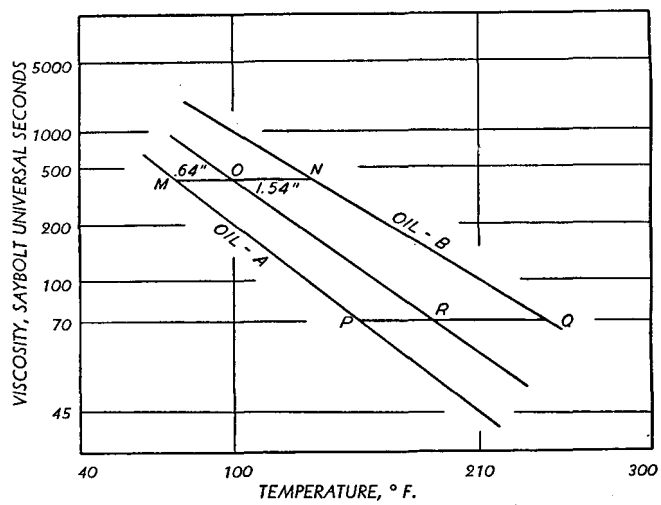

Figure 1. Use of Wright Method

1. **Draw viscosity-temperature lines of oils A and B**
2. **To make blend having 400 seconds at 100° F. use $\frac{MO}{MN} = \frac{0.64}{1.54} =$ 39.0% oil B**
3. **Locate point R so that $\frac{PR}{PQ} = \frac{MO}{MN}$ and draw viscosity-temperature line of blend through points O and R**

Table I. Properties and Sources of Oils Used in Blends

Oil	Kinematic Viscosity At 100° F.	Kinematic Viscosity At 210° F.	V.I.	A.P.I. Gravity	Description
A	45.12	5.303	22.2	22.2	Refined naphthenic oil
B	221.2	18.08	97.2	28.0	Solvent refined oil
C	30.09	4.914	92.0	30.9	Solvent refined oil
D	21.45	3.583	13.6	23.0	Raw distillate from naphthenic crude
E	392.1	16.31	−5.0	18.9	Raw distillate from naphthenic crude
F	31.92	4.419	6.8	22.8	Raw distillate from naphthenic crude
G	488.7	19.87	18.9	20.2	Raw distillate from naphthenic crude
H	187.9	9.086	−83.7	12.3	Solvent extract
J	34.21	5.612	112.7	31.8	Solvent refined oil
K	516.9	16.50	−62.5	15.4	Solvent extract
L	100.3	10.86	100.3	28.3	Acid treated paraffin base oil
M	93.81	9.691	87.5	28.3	From mixed base crude
N	116.7	9.639	52.6	19.8	Acid treated naphthenic base oil
O	312.1	19.80	77.5	24.8	Not known
P	90.52	7.312	3.9	17.1	Solvent extract blended to O.V.I.
Q	13.45	2.719	12.7	23.5	Raw distillate from naphthene base crude
R	32.21	5.190	98.9	31.1	Technical white oil

both large and small differences between viscosity indexes of the components. Viscosity ratio is defined as the ratio of the viscosity of the high viscosity oil in a system to the viscosity of the low viscosity oil, and viscosity index difference, as used in this paper, is the viscosity index of the low viscosity oil minus the viscosity index of the high viscosity oil. Table II gives percentage errors in the viscosities predicted by the various methods for the first 13 systems.

Cragoe's and Lederer's methods are modifications of the methods as they were proposed by these authors—i.e., blends were made on a volumetric rather than weight basis. In the case of the Cragoe method, the results obtained by the use of volume per cent are different from those obtained by the use of weight per cent, but both involve relatively large errors in many cases and the over-all results are about the same. Actually, a comparison based on the first eight systems shows that there is somewhat better average accuracy when volume per cent is used. In the case of the Lederer method, results are almost exactly the same for weight and volume per cent; the use of volume per cent merely results in a different numerical value of the constant.

In the above comparison, the A.S.T.M. chart and Cragoe's method gave similar results. The principal difference between the two methods is that the A.S.T.M. chart tended to give higher viscosities than the experimental ones, whereas the Cragoe method usually gave lower ones. These results are averages that include a number of somewhat extreme blends—that is, blends where the viscosity index difference is large—and therefore are not representative of the results that would be expected in normal lubricating oil blending. In fact, in the case of the A.S.T.M. chart, a considerable part of the average error can be attributed to the very large errors encountered in System JE, which has the very high viscosity index difference of 118. This error and the large errors in some less extreme systems emphasize the limitations of the A.S.T.M. chart.

Table II. Comparison of Prediction Methods

Prediction method	A.S.T.M.	Wilson	Wright	Cragoe, Vol. %	Lederer[a], Vol. %
Av. error	5.4	2.9	1.8	5.0	0.5
Max. error	25.7	10.6	10.1	13.4	1.4
% blends less than 2% in error	23	62	72	21	100

[a] Requires one experimental blend.

Table III. Viscosities of Blends

System	Composition	Kinematic Viscosity At 100° F.	Kinematic Viscosity At 210° F.	Viscosity Ratio	V.I. Difference
1. FE	25 F 75 E	179.7	11.15	12.4	11.8
	50 F 50 E	94.06	7.985		
	75 F 25 E	52.86	5.829		
2. JE	25 J 75 E	164.9	11.57	11.5	117.7
	50 J 50 E	85.13	8.598		
	75 J 25 E	51.79	6.875		
3. FB	25 F 75 B	133.5	12.68	7.0	−90.4
	50 F 50 B	82.31	8.791		
	75 F 25 B	50.74	6.250		
4. JB	25 J 75 B	130.9	13.28	6.5	15.5
	50 J 50 B	80.90	9.735		
	75 J 25 B	52.09	7.362		
5. DL	25 D 75 L	68.63	8.070	4.7	−86.7
	50 D 50 L	46.20	6.237		
	75 D 25 L	31.44	4.635		
6. CL	25 C 75 L	73.66	8.911	3.3	−8.3
	50 C 50 L	53.97	7.206		
	75 C 25 L	40.08	5.911		
7. DN	25 D 75 N	74.76	7.397	5.5	−39.0
	50 D 50 N	48.59	5.744		
	75 D 25 N	31.82	4.513		
8. CN	25 C 75 N	78.89	7.966	3.9	39.4
	50 C 50 N	54.99	6.715		
	75 C 25 N	39.86	5.690		
9. DK	25 D 75 K	183.6	10.31	24.1	76.1
	50 D 50 K	78.63	6.845		
	75 D 25 K	38.97	4.871		
10. QK	25 Q 75 K	152.2	9.408	38.4	75.2
	50 Q 50 K	58.63	5.854		
	75 Q 25 K	26.03	3.888		
11. RO	25 R 75 O	159.7	13.54	9.7	21.4
	50 R 50 O	89.09	9.596		
	75 R 25 O	52.22	6.928		
12. CO	25 C 75 O	158.2	13.46	10.4	14.5
	50 C 50 O	86.41	9.465		
	75 C 25 O	49.68	6.755		
13. QL	25 Q 75 L	60.27	7.630	7.5	−87.6
	50 Q 50 L	36.39	5.421		
	75 Q 25 L	22.00	3.825		
14. QC	25 Q 75 C	24.32	4.257	2.25	−79.3
	50 Q 50 C	20.11	3.653		
	75 Q 25 C	16.38	3.131		
15. QF	25 Q 75 F	25.29	3.892	2.36	5.9
	50 Q 50 F	20.41	3.462		
	75 Q 25 F	16.49	3.059		
16. QD	25 Q 75 D	19.11	3.362	1.59	−0.9
	50 Q 50 D	16.92	3.127		
	75 Q 25 D	15.14	2.915		
17. QJ	25 Q 75 J	27.22	4.773	2.54	−100.0
	50 Q 50 J	21.44	3.978		
	75 Q 25 J	17.02	3.249		
18. OB	25 O 75 B	238.6	18.53	1.41	19.7
	50 O 50 B	260.7	18.93		
	75 O 25 B	285.6	19.19		
19. OE	25 O 75 E	350.9	17.17	1.26	82.5
	50 O 50 E	334.7	17.81		
	75 O 25 E	321.7	18.77		
20. OG	25 O 75 G	420.8	19.78	1.56	58.6
	50 O 50 G	363.0	19.92		
	75 O 25 G	334.9	19.70		
21. OK	25 O 75 K	427.8	17.18	1.66	140.0
	50 O 50 K	371.4	17.73		
	75 O 25 K	333.9	18.81		
22. BH	(See Table IV and discussion)				
23. MP	25 M 75 P	88.62	....	1.038	−83.6
	50 M 50 P	88.56	....		
	75 M 25 P	90.56	....		
24. LP	25 L 75 P	90.52	....	1.055	−96.4
	50 L 50 P	92.09	....		
	75 L 25 P	95.17	....		
25. FC	25 F 75 C	30.14	....	1.059	85.2
	50 F 50 C	30.46	....		
	75 F 25 C	30.94	....		
26. JF	25 J 75 F	31.93	....	1.079	−105.9
	50 J 50 F	32.29	....		
	75 J 25 F	33.23	....		

The Wright method, which inherently takes into account the difference in viscosity index of the blending stocks, gives considerably better results in this comparison. The Wilson charts, or equations, give results that are better than those from the A.S.T.M. chart but not so good as those obtained when the Wright method is used. The Wright method should generally give better results than Wilson's, because the Wright method involves a quantitative measure of the difference in the oils being blended, whereas Wilson's method uses only a qualitative evaluation of the difference. Both the Wright and Wilson methods give somewhat high maximum errors, and in the case of the Wright method this seems to be an indication that there are important differences in oils which are not disclosed completely by differences in viscosity index, or more properly, by differences in viscosity-temperature slope.

The Lederer method shows an accuracy that is approximately the same as the over-all experimental accuracy. Cragoe's equation with the correction factor (Equation 5) was also employed on Systems 1 through 8 and was found to give results very similar to the Lederer method. In most cases where great accuracy is required, either Lederer's or Cragoe's correction method can be employed.

Experimental viscosities of each blend are given in Table III. Viscosities predicted by the various methods and per cent errors on each blend are not included, but are available in the original work (*15*). Four other systems were studied (*15*), but are not included because the accuracy of the work was not comparable with the results presented here.

All the viscosities so far mentioned were determined at 100° F. Viscosities at 210° F. were also determined and various prediction methods were again examined. A comparison for the first eight systems showed that errors from most of the methods were about one third as large as the errors found with viscosities at 100° F. The difference apparently can be accounted for by the decrease in viscosity ratio at 210° F., as the viscosity index difference remains the same. The decrease in the error by most of the methods is very nearly proportional to the decrease in viscosity ratio.

Table IV. System BH

Composition	Centistokes At 100° F.	Centistokes At 210° F.	V.I.
H	187.9	9.086	−83.7
10B 90H	181.3		
20B 80H	179.8	10.27	−12.3
30B 70H	179.3		
40B 60H	181.0	11.71	32.4
50B 50H	183.0		
60B 40H	187.0	13.45	63.6
70B 30H	193.0		
80B 20H	200.3	15.63	84.5
90B 10H	210.9		
B	221.2	18.08	97.2

Systems of Small Viscosity Ratio. The discussion thus far applies to systems of large viscosity ratio (1 to 13). In addition, a number of systems in which the viscosity ratio was small were investigated. It was hoped that, in such systems, the effect of viscosity ratio on blend viscosity would be minimized and that any errors resulting from the application of the various prediction methods would be almost entirely due to viscosity index difference or, at least, to some difference in the chemical nature of the oils being blended.

Systems 14 through 17 are of small viscosity ratio and are in the low-viscosity range, whereas systems 18 through 21, also of small viscosity ratio, are in a higher viscosity range. The errors from systems 14 though 17 are comparatively small by all the prediction methods investigated but the errors from systems 18 through 21 are rather large. Even Lederer's method gives an average error of 0.9% for systems 18 through 21, which is considerably more than the over-all experimental error. Investigation showed, in the case of these four systems, that the constant, *s*, in Lederer's equation did not remain essentially constant with composition. Again, in these systems, there was some indication that Wright's method failed to evaluate completely the blending characteristics of certain of the oils, as it gave results that were only slightly better than the conventional use of the A.S.T.M. chart.

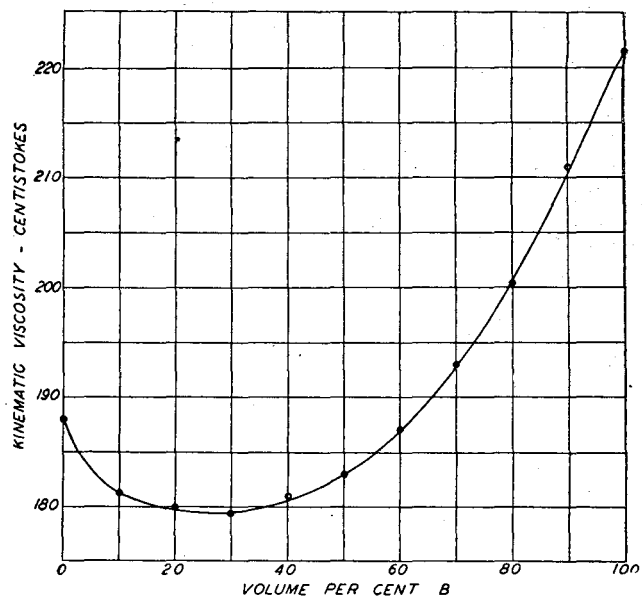

Figure 2. Viscosity of BH Blends at 100° F.

Minimum Viscosity Systems. System 22 (BH) is the most extreme of all the systems investigated. The viscosity index difference is very large (181) and the viscosity ratio is small (1.18). This system gave results that are unusual for petroleum oil systems because it exhibited a minimum viscosity. Blends were made at each 10% composition increment in order to obtain a complete viscosity-composition curve. Figure 2 is a plot of viscosities at 100° F. against composition and Table IV is a tabulation of viscosities at 100° and 210° F. for System BH. The minimum viscosity occurs at about 30% B, at which point the viscosity is 4.6% below that of oil H.

The phenomenon of minimum viscosity is well known in mixtures of certain organic compounds such as acetone–carbon disulfide (*9*), and is also found in the addition of certain salts to water (*14*), but has apparently not been very widely observed in petroleum oil systems. Only one literature reference (*7*) was found which reported similar blending characteristics—in blends of paraffin wax with transformer oil.

At 210° F., system BH shows no minimum point (see Table IV). It has been observed in some of the classical examples of minimum viscosity systems that the minimum point shifts, with increase in temperature, toward the low viscosity component and finally disappears (*9*).

For minimum viscosity to be exhibited by a system, it is generally considered that the components must be of considerably different chemical nature. It is evident, from their properties and sources, that B and H are different types of oils. That is true, even if both oils are considered to consist essentially of hydrocarbons. There is, further, the possibility that oil H may contain a considerable portion of heterocyclic ring compounds.

Further investigation was conducted in an attempt to discover additional systems exhibiting viscosity minima. Systems 23 through 26 were chosen as among the most likely to exhibit minima, because it appeared that if minima are to occur, viscosity index difference should be large and viscosity ratio small.

Of these four systems, only 23 (MP) showed a definite minimum, although the three other systems tended to exhibit a constant viscosity over certain ranges of composition.

Prediction of viscosities in systems exhibiting minima should be expected to be difficult. In general, the prediction methods discussed give viscosities that are intermediate to those of the two components. The two methods involving correction factors can be made to predict viscosity minima but only the Cragoe method gives reliable results. The Lederer method, when applied to system BH, gave very erratic results, as it showed a maximum toward B as well as the desired minimum point near H. At one point, the viscosity predicted by the Lederer equation was in error by 98%. If a prediction method is to be used on such systems, the Cragoe equation (Equation 5), or one having a similar mathematical form in which the correction factor is added or subtracted, should be used.

CONCLUSIONS

The Wright method, a modification of the conventional use of the A.S.T.M. chart, gave the best results of any of the prediction methods not requiring blend data. This conclusion is based on results from 13 systems or a total of 39 blends investigated at 100° F. and is substantiated by 12 other systems investigated at 100° F., and 8 systems investigated at 210° F. The advantage of the Wright method is probably greater in this comparison than would normally be found, because many of the systems reported here were extreme cases.

The correction methods of Lederer and Cragoe generally gave very good accuracy. Perhaps the Cragoe equation or an equation of similar form has wider application, as this type of equation was found to be fairly good even for systems having a viscosity minimum.

Certain extreme petroleum oil systems exhibit viscosity minima. For minima to occur, the oils should vary considerably in chemical nature (or viscosity index difference) and the viscosity ratio should be relatively small. There may also be other necessary conditions.

There are indications in several cases that the viscosity-temperature slope, which is used by the Wright method to evaluate the "degree of nonideality" of a system, does not always properly predict blending characteristics.

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Rapid Determination of Sulfides, Thiosulfates, and Sulfites

In Refinery Spent Caustic Solutions

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IN ORDER to avoid pollution of natural waters by alkali sulfides from refinery effluents, a close control of the sulfide content of plant spent caustic must be maintained. Rapid methods of determination are necessary, so that the air-blowing treatment that is carried out to oxidize the sulfides to thiosulfates and sulfates, which are not regarded as harmful to marine life, can be terminated at the earliest possible time. Although the sulfide content is the prime interest in plant control, it is also desirable to have a procedure that can be used to determine thiosulfates and sulfites rapidly and with a reasonable degree of accuracy.

The determination of those sulfur compounds likely to be present in caustic solution spent in oil refinery operations is described in reference texts and literature articles. The methods which are applicable to the determination of one, two, or three constituents in the mixture are usually based upon iodometric or acidimetric methods, and in the case of mercaptans (thiols), potentiometric (*11*) and amperometric (*6*) methods are given.

Sulfides may be determined by direct titration with zinc reagent (*4*), by direct iodometric titration (*12*), and by iodometric titration following precipitation from the sample with ammoniacal zinc chloride (*10*). Sulfides and thiosulfate may be determined in a mixture by removal of the sulfides with cadmium carbonate followed by iodometric determinations on both the precipitate and the filtrate (*13*). Treadwell and Hall (*12*) give methods for the determination of sodium sulfide, sodium bisulfide, and hydrogen sulfide by a combination acidimetric and iodometric method, while Scott gives a method for determining sulfides, thiosulfates, sulfites, and polysulfides in commercial sodium sulfide (*9*). Mercaptides in presence of sulfides may be determined by a potentiometric titration with silver nitrate solution (*11*).

Of these methods and numerous others, the method of Baldeschwieler (*1*) was the only one directly applicable to the analysis

A rapid volumetric method is described in which sodium sulfide, sodium thiosulfate, and sodium sulfite can be determined in caustic solution spent in oil refining processes. Treatment of an aliquot of the sample with a mixture of zinc acetate and sodium carbonate precipitates the sulfides, which may be subsequently titrated iodometrically. A portion of the filtrate is treated with formaldehyde solution, to inactivate the sulfite, and is iodometrically titrated along with an untreated portion of the filtrate. The thiosulfate content is determined from the titration carried out in presence of the formaldehyde, and the sulfite content is calculated by difference. A modified method is also presented for solutions containing mercaptides. The method is applicable to spent caustic solutions whose sulfide content is in the range 0.01 to 15.0% and may be carried out with an accuracy of approximately 3%. About 20 to 30 minutes are required for determination of the three constituents. There is included a brief discussion of the types of sulfur compounds likely to be encountered in refinery samples.

of refinery spent caustic solutions, but this procedure, while it gives accurate results, is admitted by the author to be long and tedious. It was therefore necessary to evaluate several of the existing methods and to integrate the ones most applicable into a rapid analysis procedure for refinery spent caustic solution.

DISCUSSION

Primarily the spent caustic solution (excluding "solutizer") contains sodium mercaptides, sodium sulfide, and either sodium hydroxide or sodium bisulfide; however, after oxidation either by air-blowing or by exposure to air, these compounds are either completely or partially converted to thiosulfates, disulfides, sulfites, polysulfides, sulfates, and free sulfur. The following equations for the oxidation reactions are probable (*7*):

$$12Na_2S + 9H_2O + 6O_2 \longrightarrow 18NaOH + Na_2S_2O_3 + 2Na_2S_5$$
$$2Na_2S_5 + 3O_2 \longrightarrow 2Na_2S_2O_3 + 6S$$
$$2Na_2S + 2O_2 + H_2O \longrightarrow Na_2S_2O_3 + 2NaOH$$
$$2NaHS + 2O_2 \longrightarrow Na_2S_2O_3 + H_2O$$
$$2Na_2S + 3O_2 \longrightarrow 2Na_2SO_3$$
$$4RSNa + O_2 + 2H_2O \longrightarrow 2RSSR + 4NaOH$$

Although it may appear that all these compounds may be present at various stages of oxidation, it can be shown that the coexistence in solution of certain combinations of these compounds is not likely. Sodium hydroxide and sodium bisulfide react with each other to yield sodium sulfide. Sodium sulfite reacts with sodium polysulfide in alkaline solution to yield sodium thiosulfate and sodium sulfide:

$$Na_2SX + (X - 1)Na_2SO_3 \longrightarrow Na_2S + (X - 1)Na_2S_2O_3$$

Sodium sulfite also reacts with sodium sulfide in neutral or acidic solution to give thiosulfate:

$$4NaHSO_3 + 2NaHS \longrightarrow 3Na_2S_2O_3 + 3H_2O$$

Free sulfur in alkaline sodium sulfide yields sodium polysulfide, but when the pH of this solution is lowered, free sulfur and hydrogen sulfide are formed. Mercaptans in strong sodium hydroxide are easily oxidized by air oxidation to disulfides, and at elevated temperatures the lower alkyl mercaptans, which are the ones most likely to be absorbed (*5*) in the sodium hydroxide solution if no sodium salts of fatty acids are present, decompose to sodium sulfide (*2*). In presence of free sulfur, mercaptides are converted to sulfides and disulfides (*14*).

Although it may be difficult to predict what the major components of any particular sample of caustic solution may be, so that the absence of other compounds can be assumed, certain generalizations for these compounds can be made, based upon the results of the analysis of a large number of samples. Usually the average spent caustic sample contains a small amount of free caustic, sodium sulfide, sodium polysulfide, and sodium thiosulfate. The presence of the free caustic and the polysulfides indicates that no appreciable amounts of sulfite will be encountered. The presence of the polysulfides indicates that at one time in the history of the caustic solution free sulfur was formed and this free sulfur could have reacted with the mercaptans. Furthermore, the presence of large amounts of mercaptans is not likely, as mercaptans are so easily oxidized by air in alkaline solutions. This belief was confirmed by the relatively low mercaptan content found in many samples using the method of Baldeschwieler (*1*). (Inasmuch as the determination of sulfates and disulfides was of no special interest, no attempt was made to integrate them into the scheme of analysis except to ascertain that neither interfered with the determination of the other sulfur compounds. In addition to these and the previously mentioned sulfur compounds, sodium sulfonate, which may have originated either from previous sulfuric acid treatment or from oxidation of mercaptans, may be present. The presence of small amounts of sulfonates will not affect the determinations described below.)

Initially, a procedure was developed which assumed that no mercaptides nor sulfites were present in the plant spent caustic, but it was extended to be more general, so as to encompass the eventuality that the solution could contain an excess of sulfite instead of sulfide or sulfites, sulfides, and no polysulfides. A more lengthy method was also devised to remove mercaptans if it was necessary to apply the procedure to similar solutions that might contain mercaptides.

Conventional Evolution Method of Determining Sulfides Only. The usual evolution method of determining sulfides (*8*) involves treatment of the sample with hydrochloric acid and absorption of the evolved hydrogen sulfide in ammoniacal cadmium chloride solution, from which cadmium sulfide is precipitated. The precipitate is washed, filtered, and titrated with standard iodine solution to determine the sulfide content. Preliminary experimentation indicated that this method was not applicable, because the plant caustic solution usually contained varying quantities of thiosulfate, depending upon the degree of air-blowing employed in converting the sulfides. The action of hydrochloric acid upon sodium thiosulfate generates sulfur dioxide, which reacts with the hydrogen sulfide that is being evolved and forms free sulfur. In order to eliminate this difficulty the procedure was modified so that the evolution was carried out at a pH of approximately 4.8, which is of sufficient acidity to liberate hydrogen sulfide

$$\text{Ka of } H_2S \longrightarrow (HS)^- + (H)^+ = 8.4 \times 10^{-8}$$
$$\text{Ka of } (HS)^- \longrightarrow (S)^{--} + (H)^+ = 1.2 \times 10^{-15}$$

Table I. Determination of Sulfides in Presence of Thiosulfates and Sulfites

(By modified evolution method as H_2S grams per 100 ml.)

Present	Found	Deviation, %
5.80[a]	5.54	−4.5
5.80[b]	5.43	−6.4
5.80[b]	5.60	−3.4
2.73[a]	2.62	−4.0
2.73[b]	2.59	−5.1
2.73[b]	2.65	−3.3

[a] Thiosulfate and sulfite present.
[b] Thiosulfate present.

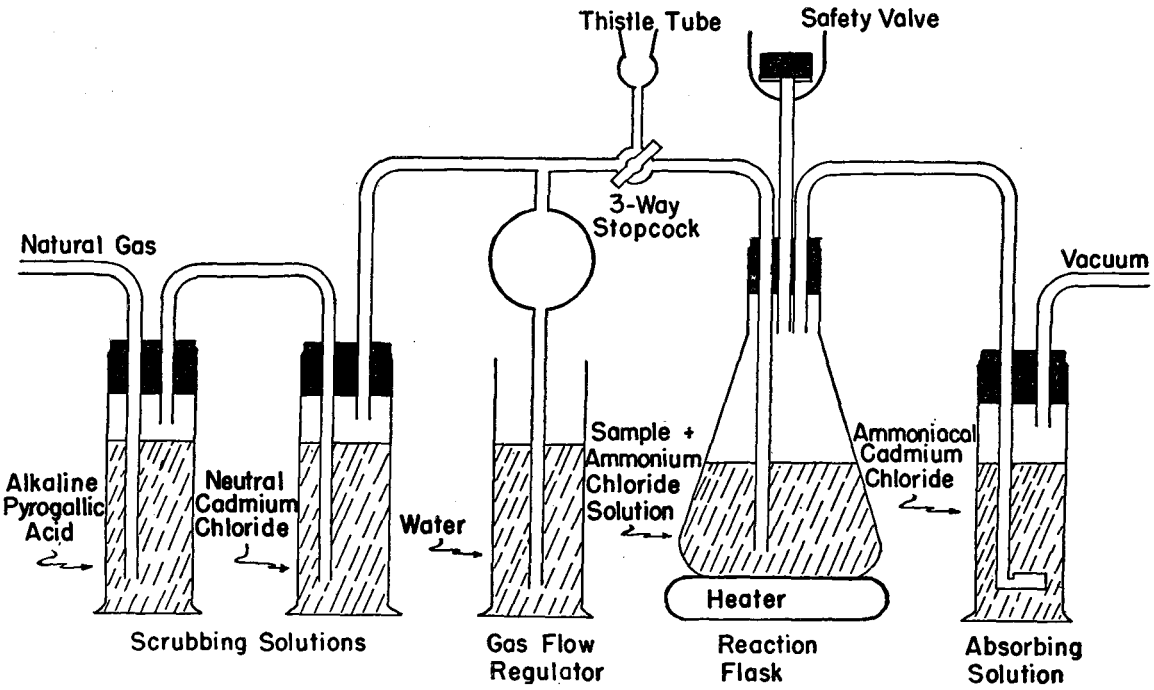

Figure 1. Apparatus for Determining Sulfides by Modified Evolution Method

from sodium sulfide but not acid enough to decompose sodium thiosulfate. This acidity can be conveniently obtained by using 15 to 20 grams of ammonium chloride per 100 ml. of water in the generating flask. When this modified evolution method was used in synthetically prepared samples containing known amounts of sulfides and thiosulfates, results that were only slightly low were obtained (Table I).

In using this method it was thought advisable to exclude air from the system, as it was feared that oxidation of the sulfide might occur before all the hydrogen sulfide could be evolved. Natural gas was passed through scrubbers containing alkaline pyrogallol solution and ammoniacal cadmium chloride before being pulled through the evolution flask as shown in Figure 1. Approximately 2 to 2.5 hours were required to carry out this determination for sulfide alone; in view of the importance of the time factor in this determination, and because multiple simultaneous determinations cannot be carried out conveniently, it was decided to concentrate on less time-consuming procedures.

SUMMARY OF PROPOSED PROCEDURES

The simplified procedure for the determination of sodium sulfide, sodium thiosulfate, and sodium sulfite is based upon the assumption that no appreciable amounts of mercaptides are present. An appropriate aliquot of the sample is treated with a mixture of zinc acetate and sodium carbonate which precipitates the sulfide, leaving the thiosulfate and sulfite unaffected in the solution. The sulfide mixed in the zinc carbonate precipitate is determined by a conventional iodometric titration. An iodometric titration is also carried out on a portion of the filtrate, to determine the total iodine titer of the thiosulfate and sulfite. The addition of formaldehyde to another portion of the filtrate will render the sulfites inactive to the iodine, so that an additional iodometric titration will serve to determine the thiosulfate content, and thus the sulfite content will also be known by difference.

If appreciable quantities of mercaptides are present, however, the above procedure must be modified to provide for the removal of the mercaptans.

The modification is based upon the precipitation of zinc sulfide from the alkaline sample, using a solution of zinc acetate that has been buffered to a pH of approximately 4.7. As the zinc sulfide may be colloidal on precipitation, it is made filterable by the addition of a small amount of gelatin which aids in flocculation (*3*). The mercaptides in the sample are converted to mercaptans by the acid solution and may be removed from the filtrate by extraction with petroleum ether, so that the thiosulfates and the sulfites may be determined without interference, as previously described.

The determination of polysulfides was of no special interest in this investigation except for their sulfide sulfur content. The polysulfides, which may be regarded as simply sulfides in which excess sulfur is dissolved, react with the zinc suspension, yielding amounts of zinc sulfide equivalent to the sulfide portion of the polysulfide molecule.

$$Na_2S_x \longrightarrow Na_2S + (X - 1)S$$

adding

$$Na_2S + Zn(C_2H_3O_2)_2 \longrightarrow 2NaC_2H_3O_2 + ZnS$$

$$\overline{Na_2S_x + Zn(C_2H_3O_2)_2 \longrightarrow 2NaC_2H_3O_2 + ZnS + (X - 1)S}$$

No method for determining polysulfides was actually investigated. However, the following procedure is recommended, should their determination be desired. This procedure is based upon the fact that polysulfides react with sulfites in neutral or basic solution to form thiosulfates.

The sample is made decidedly alkaline by the addition of sodium hydroxide, and is then divided into two equal portions. To one portion an excess of sodium sulfite is added. Both solutions are then heated to approximately 50° C. for 15 minutes. The portion containing the sulfite should be decolorized by this time, indicating the conversion of the polysulfide to thiosulfate. Both portions are treated with zinc acetate–sodium carbonate suspension to remove the sulfides. To the filtrate from the sulfite-treated portion, formaldehyde is added to form a complex with the excess sulfite. The two filtrates are then titrated with standard iodine solution, and the difference between the two represents the excess sulfur in the polysulfide now converted to thiosulfate.

REAGENTS

Iodine Solution, 0.1 *N*. Dissolve 20 to 25 grams of potassium iodide in a minimum amount of water and add 12.7 grams of

resublimed iodine. Dilute to 1000 ml. after all the iodine has been dissolved in the potassium iodine solution. Store this solution in a dark bottle to shield it from the action of light. Standardize with standard sodium thiosulfate solution or with arsenious acid.

Sodium Thiosulfate Solution. Dissolve 25 grams of sodium thiosulfate pentahydrate in 1 liter of hot distilled water that has just been boiled to remove the carbon dioxide. Add 0.1 gram of sodium carbonate to the water. When prepared in this way, the solution requires little or no aging. Standardize with standard iodine solution.

Zinc Carbonate Suspension. Dissolve 48 grams of zinc acetate dihydrate crystals and 50 grams of sodium carbonate each in 250 ml. of water. Mix the two solutions; 5 ml. of the resulting suspension will suffice for 1-ml. samples of solutions whose sulfide concentration does not exceed 5.0 grams per 100 ml.

Starch Solution. Mix 5 grams of starch in a small amount of cold water and pour this paste into 2 liters of boiling distilled water. Add 0.2 mg. of mercuric iodide to the hot solution for a preservative.

Formaldehyde, 36%.

Gelatin Solution, 0.1%. Dissolve 0.10 gram of gelatin in 100 ml. of boiling water. With 2 drops of methyl salicylate for preservative, the solution will keep several months.

Zinc–Acetic Acid Solution. Dissolve 20.0 grams of sodium hydroxide in distilled water. To this solution add 60 ml. of glacial acetic acid and 30 grams of zinc acetate (dihydrate). Dilute to 1 liter.

PROCEDURE

Short Cut Method Assuming No Mercaptides. Dilute 10 ml. of the sample volumetrically to 100 ml., making 10 ml. of the resulting solution equivalent to 1 ml. of the original sample. Pipet 10 ml. of diluted sample into a 500-ml. Erlenmeyer flask containing about 25 ml. of distilled water. Add 5 ml. of zinc carbonate suspension and shake by vigorously rotating the contents of the flask for about 5 minutes. This precipitates the sulfide as zinc sulfide. (Use 10 ml. of zinc suspension if hydrogen sulfide concentration is greater than 5.0 grams per 100 ml.) Filter off the precipitated zinc sulfide with the excess zinc carbonate (use Whatman No. 40 or S and S white ribbon) and wash the precipitating flask and precipitate four times with 20 ml. of distilled water. Combine the washing and the filtrate and reserve for use in the determination of thiosulfate and sulfite.

Table II. Determination of Sulfide, Thiosulfate, and Sulfites in Synthetic Mixtures

Sulfide			Thiosulfate			Sulfite		
H_2S, Grams/100 Ml.			$Na_2S_2O_3$, Grams/100 Ml.			SO_2, Grams/100 Ml.		
Present	Found	Deviation, %	Present	Found	Deviation, %	Present	Found	Deviation, %
A. Using Procedure Which Assumed No Mercaptides Present								
5.80	5.88	+1.6	..	..	....	...	...	...
5.80	5.88	+1.6	..	..	....	...	...	...
5.80	5.83	+0.5	..	..	....	...	...	...
5.80	5.83	+0.5	..	..	....	...	...	...
1.67	1.57	−6.3	..	..	....	...	...	...
..	..	...	5.04	5.06	+0.5	...	...	...
..	..	...	4.45	4.53	+2.0	...	...	...
..	..	...	4.48	4.57	+2.1	...	...	...
..	..	...	..	..	....	0.845	0.805	−5.0
..	..	...	..	..	....	0.749	0.716	−4.0
..	..	...	..	..	....	0.967	0.966	−0.1
5.28	5.14	−2.6	2.93	3.17	+8.2	...	...	...
5.28	5.18	−1.9	2.93	3.19	+8.9	...	...	...
1.55	1.49	−4.0	2.56	2.58	+0.8	...	...	...
1.55	1.49	−4.0	2.56	2.64	+3.1	...	...	...
..	..	...	4.75	4.73	−0.4	0.724	0.690	−4.0
..	..	...	4.75	4.75	0.0	0.724	0.740	+2.0
..	..	...	4.75	4.81	+1.2	0.724	0.686	−5.4
1.55	1.63	+5.8	2.11	2.25	+7.0	...	...	...
1.55	1.46	−5.8	2.11	2.19	+4.0	...	...	...
1.55	1.50	−3.2	2.11	2.18	+3.3	...	...	...
1.55	1.59	+2.6	2.11	2.10	−0.5	...	...	...
Av.		3.1			3.0			3.4
B. Using Procedure Which Provides for Removal of Mercaptides								Ethyl Mercaptan[a]
5.46	5.67	+3.8	..	..	....			5.0
5.46	5.17	−5.3	..	..	....			5.0
5.46	5.56	+1.8	..	..	....			5.0
5.46	5.62	+2.9	5.66	5.65	−0.2			5.0
5.46	5.62	+2.9	5.66	6.23	+10.0			5.0
5.46	5.80	+6.2	5.66	6.00	+6.0			5.0
2.35	2.40	+2.1	2.89	3.03	+4.8			2.5
2.35	2.43	+3.4	2.89	3.07	+6.2			2.5
2.35	2.28	−3.0	..	..	....			2.5
Av.		3.5			3.6			

[a] Grams per 100 ml. present.

Table III. Reproducibility of Sulfide Determination Multiple Determinations on Single Sample

H_2S Found, Grams/100 Ml.	Deviation from Mean	Deviation[2]
4.01	+0.09	0.0081
3.97	+0.05	0.0025
3.86	−0.06	0.0036
3.89	−0.03	0.0009
3.76	−0.16	0.0256
3.76	−0.18	0.0256
3.97	+0.05	0.0025
4.05	+0.13	0.0169
3.90	−0.02	0.0004
3.99	+0.07	0.0049
Mean = 3.92	Σd = 0.82	Σd^2 = 0.0910

Average deviation = 0.082 absolute or 2.1% relative
Standard deviation = 0.101 absolute or 2.6% relative
Probable deviation of single determination = 0.058 absolute or 1.5% relative

In the empty 500-ml. Erlenmeyer flask which was used to precipitate the zinc sulfide, place about 25 ml. of distilled water, a measured amount of 0.1 *N* iodine solution, and approximately 10 ml. of 1 to 1 hydrochloric acid. If the sulfide content is about 7.5 grams per 100 ml., use 50 ml. of standard iodine solution; if it is 4 grams per 100 or lower, use 25 ml. To the diluted and acidified iodine solution add the filter paper containing the zinc sulfide and zinc carbonate. Swirl the contents to ensure a complete reaction between the iodine and the zinc sulfide. Back-titrate with standard thiosulfate until the iodine turns from brown to yellow. Add 5 ml. of starch solution and continue titrating with the thiosulfate until the blue color disappears.

Divide the filtrate containing the thiosulfates and sulfites into equal or aliquot parts. To a measured volume of standard iodine solution, acidified with approximately 3 ml. of acetic acid, add one portion of the filtrate and back-titrate with sodium thiosulfate, using starch as an indicator. To another measured volume of standard iodine solution acidified with acetic acid, add the remaining portion of the filtrate and 5 ml. of 37% formaldehyde, and titrate with sodium thiosulfate solution as before. The formaldehyde combines with and inactivates the sulfite while the thiosulfate is not affected. Therefore the difference in the two titrations represents the sulfite content.

Method Providing for Removal of Mercaptides. Dilute 10 ml. of sample to 100 ml., making 10 ml. of the resulting solution equivalent to 1 ml. of the original sample. Slowly add a 10-ml. aliquot of the sample dropwise with constant stirring into 30 ml. of zinc acid solution. Filter, using a suction flask, and wash four times with water and twice with isopropyl alcohol. Discard the alcohol washings, but retain the filtrate and the water washings for subsequent determination of thiosulfate and sulfite. Add the precipitated zinc sulfide with the filter pulp to 50 ml. of 0.1 *N* iodine solution acidified with 10 ml. of 1 to 1 hydrochloric acid. (This amount of iodine is equivalent to 80 mg. of sulfide sulfur.) Swirl the content to ensure a complete reaction between the iodine and the zinc sulfide. Back-titrate with standard sodium thiosulfate solution until the iodine turns from brown to yellow, then finish the titration, using starch as the indicator. Add 100 ml. of petroleum ether to the filtrate and washings, which was reserved for the thiosulfate and sulfite determination, and place in a 250-ml. separatory funnel. Remove the mercaptans with three successive extractions with petroleum ether, discarding the hydrocarbon layer each time. Carry out the determination of thiosulfates and sulfites on the aqueous portion in a manner identical to that previously described.

Evaluation. Solutions containing known concentrations of sodium sulfide, sodium sulfite, sodium thiosulfate, and various combinations of these solutions in the presence and in the absence of sodium ethyl mercaptide were analyzed by the procedures to check their accuracy and reproducibility. It may be seen from Table II that an average accuracy of ±3 to 4% was obtained

on the sulfide, thiosulfate, and sulfite determinations using both methods. A reproducibility study was made on the sulfide determination (in absence of mercaptides) by carrying out the determinations on a single sample. In Table III it may be seen that the probable deviation of a single determination is 1.5% based on the average amount of sulfide found. The simplified procedure requires approximately 20 to 30 minutes for the analysis of a single sample; the procedure providing for the removal of the mercaptans will require a somewhat longer amount of time, depending upon how rapidly the zinc sulfide will filter, and this, in turn, is contingent upon the success the analyst has in precipitating the zinc sulfide in a crystalline state. Multiple determinations can easily be carried out simultaneously by either procedure.

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p-Aminobenzoic Acid and Its Sodium Salt

Properties of Analytical Interest

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Properties of highly purified *p*-aminobenzoic acid and its sodium salt were investigated as possible additional criteria of purity and as the basis of new methods for determination of *p*-aminobenzoic acid after suitable preliminary separation. When *p*-aminobenzoic acid is titrated with a strong base, a sharp end point is found between pH 6.5 and 8.5. On titrating sodium *p*-aminobenzoate with a strong acid one obtains a useful equivalence point at pH 3.5. A characteristic absorption spectrum may be obtained in isopropyl alcohol, wave length maximum = 288 mμ $E^{1\%}_{1\,cm.}$ = 1370. In water both *p*-aminobenzoic acid and its sodium salt show about the same characteristic wave length, maximum = 266 mμ and $E^{1\%}_{1\,cm.}$ = 1070. Beer's law is obeyed in both isopropyl and aqueous solutions. In a comparison of the spectrophotometric, titrimetric, and diazo methods, the new method showed up favorably. All three measurements are suggested for the complete characterization of pure *p*-aminobenzoic acid.

PARA-aminobenzoic acid (*2*) has gained importance in recent years because of its physiological properties. It may be considered a member of the vitamin B complex (*1, 7*). The evidence in the literature indicates that sulfa drugs act as an antibiotic in competition with *p*-aminobenzoic acid in the microorganism (*11, 12, 15, 16*). Successful treatment of the Rickettsia disease has increased interest in this compound (*13*). Furthermore, it appears to be important as a means of maintaining high salicylate levels in the treatment of rheumatic fever (*6*).

The reasons cited above indicate the importance of additional criteria for the purity of *p*-aminobenzoic acid and its sodium salt. These properties may be also useful in its determination in various products after suitable preliminary separation.

The sodium salt of *p*-aminobenzoic acid was purified by the addition of sufficient C.P. sodium hydroxide to convert *p*-aminobenzoic acid to its sodium salt, treated three times with charcoal, and crystallized three times from aqueous solution. The salt was washed three times with 95% alcohol and dried at 100° C.

To prepare purified *p*-aminobenzoic acid, the sodium salt was redissolved in distilled water and enough C.P. hydrochloric acid was added to convert it to the *p*-aminobenzoic acid. The purified substance was washed with water until free of chlorides and dried at 100°. The *p*-aminobenzoic acid thus purified melted sharply at 187° C. and other batches melted at 187° to 187.5° C., compared to 186° to 188° C. previously reported for the purified product (*3, 4*)

The pH changes during titration of *p*-aminobenzoic acid with a standard alkali are given in Figure 1. There is the characteristic sharp change in pH beginning at about 6.5, which is desirable in a good titrimetric reaction. The pH of the equivalence point is 7.85. Thus an indicator that changes color between 7.0 and 8.7 is suitable for this titration. The pKa obtained is 4.65 and 4.80, which may be compared to 4.8 (*4*) and 4.68 (*3*). (The pKa was obtained from the relationship pKa = pH at one-half neutralization of weak acid.) The titration curve of sodium *p*-aminobenzoate, given in Figure 2, is essentially the reverse of that found for *p*-aminobenzoic acid. The pH of the sodium salt is 7.86 and when treated with one equivalent of hydrochloric (or sulfuric) acid the pH is 3.50. The change in pH at the end point is not so sharp as for the acid. Nevertheless one may obtain results of high precision when titrating to this pH. The high precision that may be

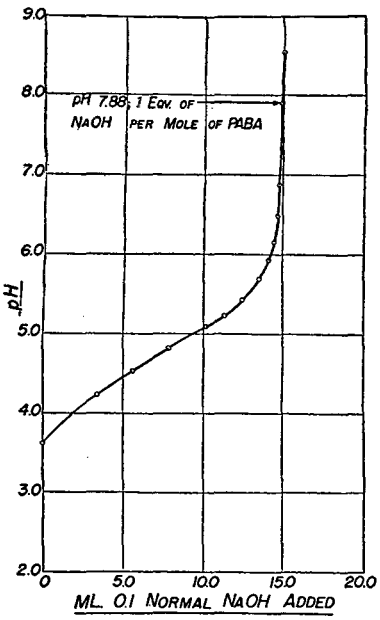

Figure 1. pH Changes during Titration of *p*-Aminobenzoic Acid (205.0 Mg. in 100 Ml. of Water) with 0.1 *N* Sodium Hydroxide

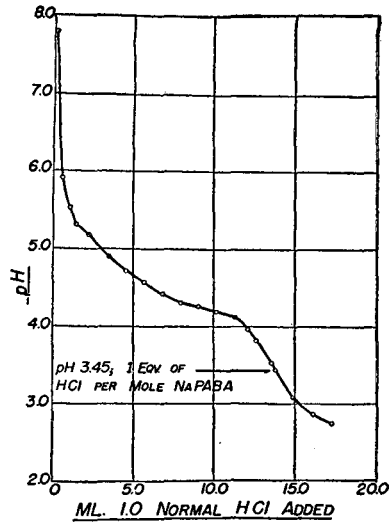

Figure 2. pH Changes during Titration of Sodium *p*-Aminobenzoate (2194.8 Mg. in 100 Ml. of Water) with 1.0 *N* Hydrochloric Acid

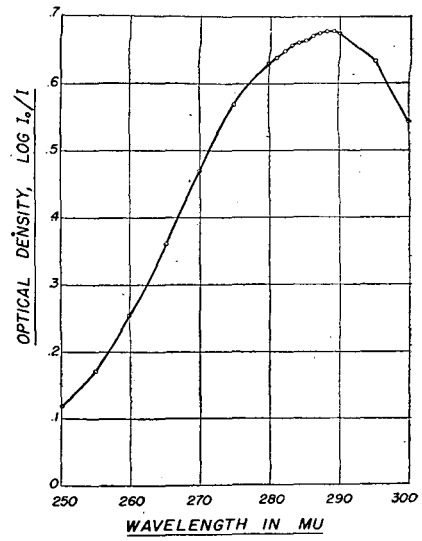

Figure 3. Absorption Spectrum of Pure *p*-Aminobenzoic Acid in 99% Isopropyl Alcohol

503.4 micrograms per 100 ml. 1.0-cm. light absorption path

obtained by titration for both *p*-aminobenzoic acid and its sodium salt is indicated in Table I.

It is evident that most impurities would change the magnitude of titration of both products—for example, titrimetrically inert material (such as water, sand, etc.) would decrease the magnitude of the expected titration. In contrast, titrimetrically active impurities would cause a higher or lower titration, depending on the equivalent weight of the contaminant. Even when the concentration of impurities is such that owing to compensation of errors the magnitude of titration is the same, the shape of the titration curve would change in most cases. Isomers of *p*-aminobenzoic acid may be eliminated by comparison of the shape of the absorption spectrum (*8*) and the magnitude of the extinction coefficient at the wave length of maximum absorption given in Figures 3 and 4 and Table II.

Figure 3 shows the absorption spectrum of *p*-aminobenzoic acid in isopropyl alcohol, which was chosen because this compound is fairly soluble in this solvent. Wave-length maximum is 288 to 289 mμ, $E^{1\%}_{1\ cm.}$ = 1365; ε = 18,771 (see Table II). These values may be compared to those reported by Kumler (*9*), who found wave-length maximum = 288 mμ and ε = 17,400 in 95% commercial ethanol, and by Doub and Vandenbelt (*5*), who found wave-length maximum = 284 and ε = 14,000 in water at pH 3.75. The values of Kumler are somewhat higher than those obtained by the present authors in 95% alcohol as shown in Table II, but lower than those in isopropyl alcohol. The values of Doub and Vandenbelt (*5*) in water are lower than those obtained by the authors in water for *p*-aminobenzoic acid but values for the sodium salt (ε = 14,900, maximum = 265) compare well with the authors' for both products in water.

Figure 4 shows the relation between concentration of *p*-aminobenzoic acid in isopropyl alcohol and optical density at

Table I. Comparison of Diazo with Titrimetric and Spectrophotometric Methods of Determining Pure *p*-Aminobenzoic Acid and Its Sodium Salt

(Values expressed in % of theoretical value)

Sample	Melting Point, ° C.	Method: Titrimetric with 0.1 *N* H_2SO_4	Method: Spectrophotometric	Method: Diazo (*8*)
I Sodium PABA	.....	99.93	100.18	99.35
II Sodium PABA	.....	100.17	Used as standard	99.94
III Sodium PABA	.....	100.29	100.18	99.64
IV PABA	187–187.5	With 0.1 *N* NaOH 100.29	100.5	99.08
V PABA	187–187.5	100.64	100.4	99.35
VI PABA	187.0	100.46	Used as standard	99.12

Table II. Extinction Coefficient and λ Maxima

Product	Solvent	λ Max.	$E^{1\%}_{1\ cm.}$		pH	PABA, Mg. %
PABA 5	Isopropanol 99%	288–290	1380	18,910	...	0.5035
PABA 6	Isopropanol 99%	288–289	1343	18,400	...	0.5034
PABA (no caustic treatment)	Isopropanol 99%	288–289	1349	18,490	...	0.5032
PABA 5	Purified isopropanol 99%	289–290	1385	18,980	...	0.5032
PABA 6	Purified isopropanol 99%	288–289	1355	18,570	...	0.5025
PABA 5	Commercial ethanol 95%	287–288	1186	16,250	...	0.5041
PABA 6	Commercial ethanol 95%	287–288	1151	15,760	...	0.5041
PABA 5	Distilled water	266	1088	14,900	...	0.4008
PABA (no caustic treatment)	Distilled water	266	1093	14,980	...	0.4070
Sodium PABA 2	Distilled water	266	1078[a] 929	14,770	...	0.4027[b] 0.3470
Sodium PABA 3	Distilled water	266	1072[a] 924	14,690	...	0.4047[b] 0.3487
PABA 5	Distilled water	266	1099	15,060	3.59	0.4038
PABA 5	Distilled water and 1/2 equivalent of NaOH	266	1102	15,090	4.78	0.4021
PABA 5	Distilled water and 1 equivalent of NaOH	266	1092	14,960	9.19	0.4040
PABA 5	Distilled water and 2 equivalents of NaOH	266	1088	14,900	11.58,	0.4027

[a] Calculated on PABA content in Na PABA.
[b] In terms of mg. % Na PABA.

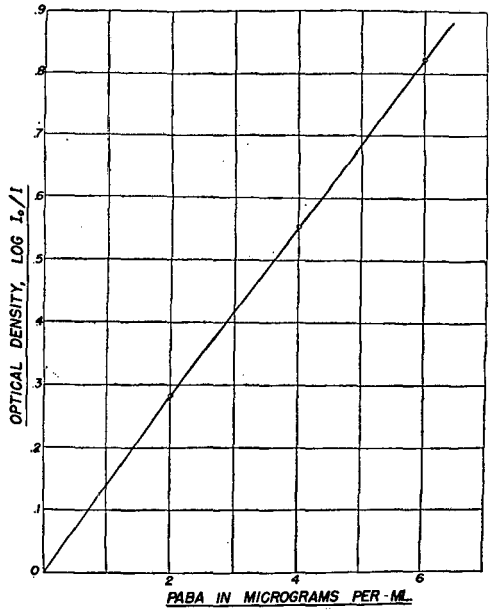

Figure 4. Relation between Absorption at Maximum (288 mμ) and *p*-Aminobenzoic Acid Concentration in 99% Isopropyl Alcohol

1.0-cm. light absorption path

288 mμ (which is the wave length of maximal absorption). As may be readily observed, Beer's law holds and thus the quantitative usefulness of this set of conditions is indicated.

Figure 5 shows the absorption spectrum of the sodium salt of *p*-aminobenzoic acid in water; wave-length maximum = 266 mμ; $E^{1\%}_{1\text{ cm.}} = 1075$ (based on *p*-aminobenzoic acid content); $\epsilon = 14{,}730$. This shift in the maximum to the further ultraviolet is apparently due to the solvent and not to use of the sodium salt, as shown by the fact that *p*-aminobenzoic acid treated with various amounts of sodium hydroxide (see Table II) gave the same maxima and identical absorption spectra. This observation appears to be at variance with those of Doub and Vandenbelt (*5*) and of Kumler and Strait (*10*), who indicate that there is a shift in the maximum from 284 to 266 mμ due to 0.1 *N* sodium hydroxide (*5*) or 1 *N* sodium hydroxide (*16*) rather than the solvent. Furthermore, these authors indicate that the extinction coefficient of the sodium salt should be higher, whereas in this study it appears essentially the same as that of *p*-aminobenzoic acid in water.

The difference may be due to the small amount of alcohol used by these authors to facilitate solution (*5*). However, when *p*-aminobenzoic acid was dissolved in methyl alcohol up to 7% of the final volume, the maximum and extinction coefficient were the same as in water. The sodium salt when dissolved in isopropyl alcohol (in which it is only slightly soluble) showed a maximum from 268 to 270 mμ. It is possible therefore that the above workers (*5*, *10*) obtained this shift in alcoholic solution rather than in water.

The sodium salt obeys Beer's law in water, as shown in Figure 6. Thus the quantitative usefulness of these conditions is indicated again.

The extinction coefficients of both *p*-aminobenzoic acid and its sodium salt are of a sufficiently high magnitude to permit the determination of 1 to 2 micrograms per milliliter of solution, with a high degree of precision (optical density being about 0.1 to 0.2 for 1 to 2 micrograms per ml.).

The high degree of precision that may be obtained when the ultraviolet absorption maxima are employed in isopropyl alcohol for *p*-aminobenzoic acid and in water for the sodium salt is shown in Table I. Here both the spectrophotometric and titrimetric methods are compared to the U.S.P. method in which *p*-aminobenzoic acid is titrated by diazotization (*14*) and depends on the amino group present.

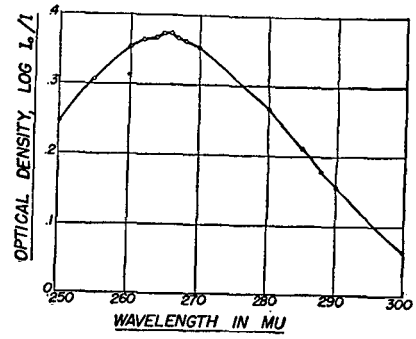

Figure 5. Absorption Spectrum of Pure Sodium *p*-Aminobenzoate in Distilled Water

402.7 micrograms per 100 ml. 1.0-cm. light absorption path

EXPERIMENTAL

The spectrophotometric data were obtained with a Beckman quartz spectrophotometer, Model DU, equipped with the ultraviolet accessory set. The wave-length scale setting was checked using several of the hydrogen lines emitted by the hydrogen discharge lamp.

The pH data were compiled using a Leeds & Northrup potentiometer, Catalog No. 7661, and a Beckman pH meter, Model G.

The *p*-aminobenzoic acid samples were assayed by titrating accurately weighed samples, dissolved in distilled water, with 0.1 *N* sodium hydroxide, using phenolphthalein test solution as the indicator. One milliliter of 0.1 *N* sodium hydroxide is equivalent to 13.71 mg. of *p*-aminobenzoic acid.

The sodium salt samples were assayed by potentiometrically titrating accurately weighed samples, dissolved in distilled water with 0.1 *N* sulfuric acid until a pH of 3.45 was reached. One milliliter of 0.1 *N* sulfuric acid is equivalent to 15.9 mg. of sodium *p*-aminobenzoate.

Samples of both *p*-aminobenzoic acid and its sodium salt were assayed spectrophotometrically through the use of selected samples of each which were used as standards.

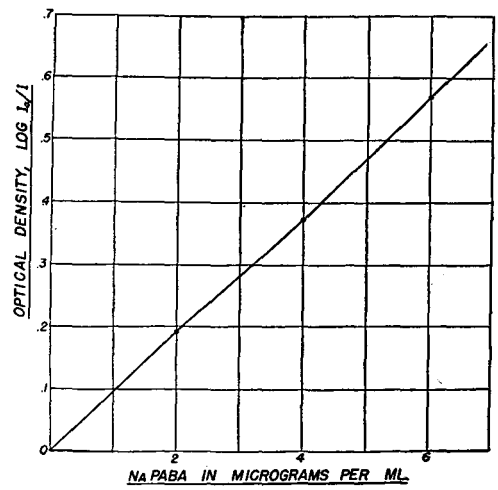

Figure 6. Relation between Absorption at Maximum (266 mμ) and Sodium *p*-Aminobenzoate Concentration in Distilled Water

1.0-cm. light absorption path

Procedure I. An accurately weighed sample is transferred to a volumetric flask with 99% isopropanol. Dilutions are further made in volumetric flasks so that a 1-ml. aliquot of the final solution will contain from 0.0002 to 0.00025 gram of *p*-aminobenzoic acid. One milliliter of this stock solution is further diluted to 100 ml. and is spectrophotometrically assayed at a wave length of 288 mμ. This step is also carried out using 2-, 3-, 4-, and 5-ml. aliquots of the stock solution which are diluted to 100 ml. These solutions are also spectrophotometrically assayed and the density readings obtained are plotted against the milliliters taken from the stock solution. This curve when plotted is linear.

The sample to be assayed is treated in a similar manner to the standard; the dilutions were calculated to show a reading approximately in the center of the plotted curve. The density

reading obtained, when plotted against the standard curve, gives the per cent purity of the *p*-aminobenzoic acid.

Procedure II. Sodium *p*-aminobenzoate may be assayed in a similar manner, except that the diluent used is distilled water and spectrophotometric determinations are made at wave length 266 mμ.

ACKNOWLEDGMENT

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Colorimetric Determination of Certain Alpha, Beta-Unsaturated Aldehydes

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***m*-Phenylenediamine dihydrochloride is a specific color reagent under the proper conditions for α,β- unsaturated aldehydes and ketones in addition to a few other highly reactive aldehydes. Advantage has been taken of this specificity in developing successful procedures for the quantitative determination of cinnamaldehyde, crotonaldehyde, and furfural without interference from such common impurities as acetaldehyde and benzaldehyde.**

DURING an investigation in this laboratory, it became necessary to develop a method for determining cinnamaldehyde in the presence of benzaldehyde and acetaldehyde, which are the usual accompanying impurities. No method was available from the literature, as procedures based on the use of such reagents as hydroxylamine, fuchsin–sulfurous acid, sodium bisulfite, semicarbazide, thiosemicarbazide, and semioxamazide showed little or no selectivity in their reactions. Feigl (*3*) reveals that the colored Schiff base derived from *o*-dianisidine and cinnamaldehyde is detectable in extremely small concentrations, whereas the corresponding benzaldehyde and acetaldehyde reactions are much less sensitive. However, preliminary investigation showed that the color was unstable and not suitable for adoption as a quantitative method. Fellenberger (*4*) reported a method for the determination of cinnamaldehyde based on a color reaction with sulfuric acid and isobutyl alcohol, but it was not investigated because the color tone was said to vary with dilution.

A note on the A.O.A.C. colorimetric method (*1*) for the determination of citral as originally reported by Hiltner (*5*) revealed that ethyl alcohol could be used as solvent without purification, as the usual impurity, acetaldehyde, did not interfere under the conditions of the determination. This method is based upon Schiff base formation between *m*-phenylenediamine dihydrochloride and citral, giving a highly sensitive yellow to orange coloration in 80% aqueous alcohol containing oxalic acid. The inclusion of the oxalic acid in the reagent to obtain more uniform colors in the presence of easily oxidizable terpenes and the use of a blue 420 mμ filter in conjunction with a photoelectric colorimeter to compensate for dyes present in some lemon and orange extracts have been discussed (*2*, *6*).

During the course of this investigation, an abstract of a recent paper by Wachsmuth and Lenaers (*8*) was noted, which described a colorimetric method for the determination of cinnamaldehyde in cinnamon essence and extracts based upon a reaction with *p*-phenylenediamine in acetic acid solution. Hiltner (*5*, *6*) had reported earlier that reproducibility of results with this amine in the determination of citral had been difficult, and that *m*-phenylenediamine was a superior reagent.

Because of the reported specificity of this reagent for citral in the presence of acetaldehyde, it was decided to investigate its use for the determination of cinnamaldehyde.

In a preliminary evaluation of the selectivity of the reagent solution as prepared by the A.O.A.C. directions, a large number of aldehydes and ketones in concentrations approximately 10 to 20 times that required to produce a strong color with citral were tested. When no color developed at room temperature, the solution was heated at 60° to 65° C. in a water bath for 15 minutes (Table I). It is apparent that the sensitivity of the reaction varies directly with the general reactivity of the aldehydes and ketones. Strong positive reaction was given by α,β- unsaturated aldehydes and ketones and by highly reactive aromatic aldehydes such as vanillin. Ordinary aliphatic and simple aromatic aldehydes as well as ordinary ketones (including methyl and methyl aryl ketones) did not react even on heating. The only borderline cases were 2-ethylhexaldehyde, cyclamal, and isobutyraldehyde, in which the formyl group is apparently more reactive on account of its attachment to a secondary carbon atom. A postulation that the reagent is not specific for citral and possibly applies generally to α,β- unsaturated aldehydes has just appeared in a paper by Price and Dickman (*7*), on a study of the cyclization of citral and citronellal. Their prediction is in good agreement with the work presented here.

These results indicate the possibility of adapting the reaction to the colorimetric determination of a number of relatively reactive aldehydes and ketones without interference from the

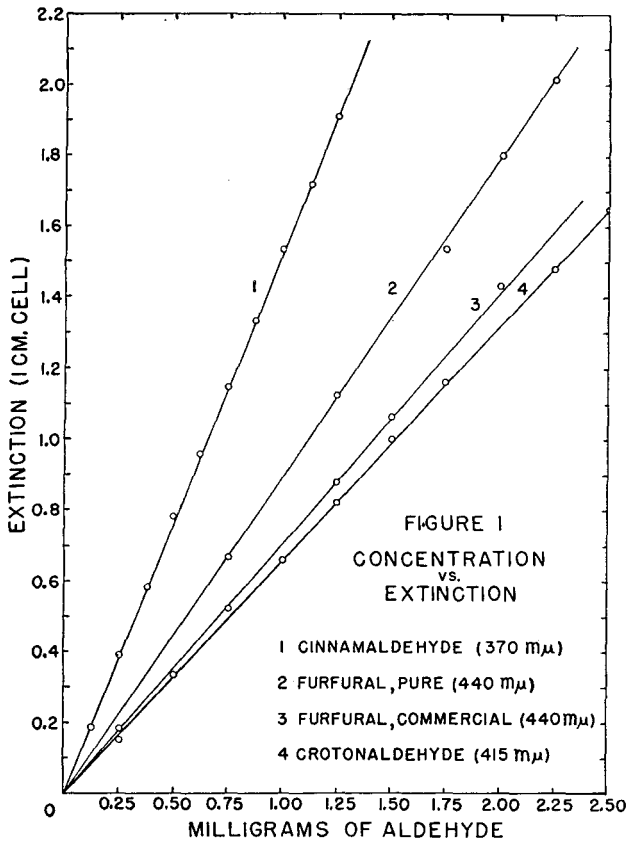

FIGURE 1
CONCENTRATION
vs.
EXTINCTION

simple aromatic and aliphatic aldehydes and ketones. The successful use of the reagent for the determination of cinnamaldehyde, crotonaldehyde, and furfural is described in detail below.

APPARATUS AND SOLUTIONS

Instrument. Spectrophotometric measurements were made on a Beckman quartz spectrophotometer, Model DU, using 1.00-cm. Corex glass cells. Any standard photoelectric colorimeter used with properly selected filters would be satisfactory.

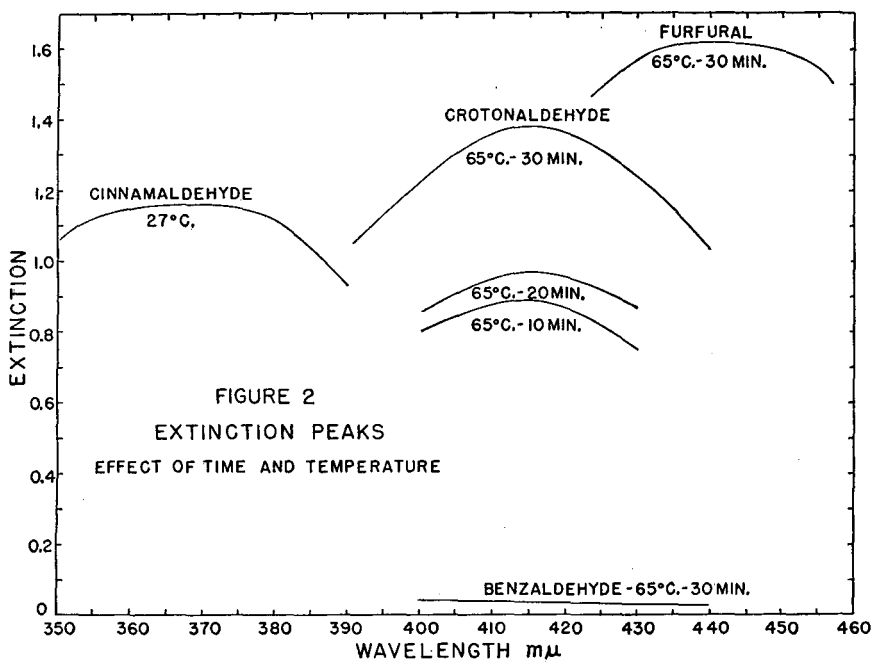

FIGURE 2
EXTINCTION PEAKS
EFFECT OF TIME AND TEMPERATURE

Table I. Reaction of Aldehydes and Ketones with *m*-Phenylenediamine Dihydrochloride–Oxalic Acid Reagent

Compound	Type	Reaction: Cold	Reaction: Heated
Citral	α,β-unsaturated aldehyde	+	..
Cinnamaldehyde		+	..
Acrolein		+	..
Furanacrolein		+	..
Thienyl acrolein		+	..
Acetaldehyde	Aliphatic aldehyde	−	−
Formaldehyde	.....	+	+
Isobutyraldehyde	Secondary aliphatic aldehyde	−	+
2-Ethyl hexaldehyde		−	+
Cyclamal	C_6H_5-substituted aliphatic aldehyde	−	+
Chloral	Substituted aliphatic aldehyde	−	−
Pyruvic aldehyde	α-Keto aliphatic aldehyde	−	+
Benzaldehyde	Aromatic aldehyde	−	−
Cuminic aldehyde	Substituted aromatic aldehyde	+	..
Vanillin		+	..
p-Hydroxybenzaldehyde		+	..
o-Methoxybenzaldehyde		+	..
Mesityl oxide	α,β-unsaturated ketone	+	..
Isophorone		+	..
Methyl vinyl ketone		+	..
Carvone	α,β-unsaturated ketone (cyclic)	−	+ (slight)
Benzophenone	Aromatic ketone	−	−
Diethyl ketone	Aliphatic ketone	−	−
Acetone	Methyl ketone	−	−
Acetophenone	Methyl aryl ketone	−	−
Glyoxal	...	+	..

Reagent Solution. Prepare *m*-phenylenediamine dihydrochloride–oxalic acid solution, according to the standard A.O.A.C. method (*1*). Prior to use purify commercial *m*-phenylenediamine dihydrochloride (Eastman white label grade) by digesting 25 grams for 5 minutes in 75 ml. of alcohol three times, removing the solvent each time by decantation. Dry crystals on a steam bath or in a drying oven.

Dissolve 2.500 grams of dried crystals in 37.5 ml. of distilled water and dissolve 2.500 grams of crystallized oxalic acid in 50 ml. of absolute alcohol. Mix the two solutions and filter if not clear. Transfer to a 250-ml. volumetric flask and dilute to the mark with absolute alcohol. This solution decomposes easily and should be made fresh at the first sign of sediment formation.

The quality of several lots of *m*-phenylenediamine dihydrochloride which were purchased during the course of this work appeared to be somewhat variable. It is advisable to check the calibration curves whenever a new bottle is used.

Standard Solution. Prepare a standard solution from each of the aldehydes after purification by fractional distillation, by dilution with absolute alcohol to a concentration of 0.0250 mg. per ml. of solution for crotonaldehyde and furfural, and 0.1250 mg. for cinnamaldehyde.

DETERMINATION OF CINNAMALDEHYDE

Procedure. Weigh an amount of sample to contain 0.125 to 1.250 mg. of cinnamaldehyde, transfer to a 50-ml. volumetric flask, add 5.00 ml. of reagent, and dilute to the mark with absolute alcohol. Measure the extinction in a 1.00-cm. cell at 370 mμ exactly 1.5 minutes after pouring the reagent into the flask, using in the comparison cell a blank containing 5.00 ml. of reagent diluted to 50 ml. with absolute alcohol. This blank is prepared just prior to the sample. The calibration curve thus obtained is shown in Figure 1. The percentage of cinnamaldehyde in unknowns is read from this curve or determined from the equation: mg. of cinnamaldehyde = $0.65 \times E$.

The maximum extinction was found by experiment to be 370 mμ, and all measurements were made at this wave length (see Figure 2).

A strict time schedule must be followed in making the measurements. The intensity of the color remains constant for approximately 3 minutes after the reagent is poured in, after which it gradually fades at the rate of 2 to 3% of the reading per minute. Heating the reaction mixture only accelerates the fading, and although a point is reached where fading becomes very slow, no reproducible intensity can be obtained in this manner. As the addition of larger quantities of reagent causes progressive increases in intensity without leveling off, the reagent must be added from a pipet.

Several points on the calibration curve were checked on different days with freshly prepared samples and blanks and with new reagent solutions, with the result that reproducibility was regularly obtained within an average of ±1%.

At 370 mμ, benzaldehyde and acetaldehyde caused no interference with the determination.

This method has been employed with excellent results for numerous analyses where mixtures of essential oils containing cinnamaldehyde were first recovered from various flavored products by steam distillation.

Table II. Effect of Temperature and Duration of Heating Period on Development of Color with Crotonaldehyde

Temperature of Bath, ° C.	Time of Heating, Min.	Extinction (Measured at 415 mμ), after Cooling before Measurement for: 2 min.	7 min.	30 min.	60 min.
75	10	0.800	0.798	0.840	0.880
60–65	20	0.888	0.884	0.920	...
60–65	30	1.37	1.37	1.37	1.38
60–65	40	1.64	1.63	1.65	1.65
60–65	50	1.71	1.71	1.71	1.71
60–65	60	1.82	1.83	1.85	...

DETERMINATION OF CROTONALDEHYDE AND FURFURAL

Satisfactory modifications of the above method have been developed for crotonaldehyde and furfural. Heat was found to be necessary to develop a stable color, and a few other minor adjustments were required.

Procedure for Crotonaldehyde. Weigh an amount of sample to contain 0.25 to 2.5 mg. of aldehyde, transfer to a 50-ml. volumetric flask, add 15.00 ml. of reagent, and dilute to the mark with absolute alcohol. Transfer most of the solution to a 100-ml. flask with a ground-glass stopper and heat at 65° C. for 30 minutes in a water bath. (Heating is done in a large flask to simplify closure and to minimize evaporation.) Cool by placing in an ice bath for 1 minute. Prepare a blank by diluting 15.00 ml. of reagent to 50 ml. with absolute alcohol. Heat and cool it in the same manner and at the same time as the sample. Place the cooled sample in a 1.00-cm. cell and measure the extinction at 415 mμ, using the blank in the comparison cell. The samples must be measured within 30 minutes after cooling. The crotonaldehyde content of unknowns may be determined by reference to the calibration curve (Figure 1) or by calculation from the equation: mg. of crotonaldehyde = $1.52 \times E$.

Procedure for Furfural. The procedure for furfural is the same as that for crotonaldehyde, except that 25.00 ml. instead of 15.00 ml. of reagent are used and the extinction must be measured within 3 minutes after cooling. Measurement in this case is made at 440 mμ.

The calibration curve is presented in Figure 1, and the equation is: mg. of furfural = $1.10 \times E$. Figure 1, curve 3, illustrates the validity of the Beer-Lambert law even when a tarry sample of commercial furfural is used.

Effect of Temperature and Duration of Heating Period. Unlike cinnamaldehyde, both crotonaldehyde and furfural are slow in developing color after addition of the reagent; it is therefore necessary to heat the solution to hasten development of a stable color. Table II shows this effect clearly.

Thus a color stable for at least 30 minutes was obtained with a heating period of 30 minutes at 60° to 65° C. The same conditions were found to be suitable for the determination of furfural except that the color measurement had to be made within 3 minutes after cooling, as there was a tendency for the intensity to increase very slowly after 3 to 5 minutes.

Table III. Effect of Reagent Concentration in Crotonaldehyde and Furfural Determinations

Compound Determined	Reagent Solution Added, Ml.	Extinction after Cooling before Measurement for: 1.5 min.	2 min.	2.5 min.	3 min.	5 min.	10 min
Furfural	5	0.385	0.389	0.390	0.392	0.397	0.407
	10	0.800	0.802	0.803	0.803	0.805	0.812
	20	1.06	1.06	1.06	1.07	1.08	1.14
	25	1.62	1.62	1.62	1.63	1.65	1.60
Crotonaldehyde	2	...	0.778	...	...	0.778	0.776
	5	...	1.37	...	...	1.37	1.37
	10	...	...	...	...	1.72	...
	15	1.76	...	...	...	1.76	...
	20	...	...	...	...	1.76	...

Effect of Concentration of Reagent. A study was made of the optimum concentrations of the *m*-phenylenediamine dihydrochloride reagent for both crotonaldehyde and furfural (Table III). Considerable excesses of oxalic acid over the amount called for in the reagent solution were found to have no effect on the color development.

Reproducibility of Results. Using the standardized proce dures, good results were obtained on numerous analyses. For example, from a group of eight checks run on crotonaldehyde over a period of one month with several different reagent solutions, the greatest error observed was ±1.7%, and the average was easily within 1%.

Extinction Peaks of Developed Colors. The point of maximum absorption was determined for each aldehyde studied. These curves, presented in Figure 2, were the basis for the selection of the wave length at which color measurement was made.

Interferences. As reported in the original A.O.A.C. method, acetaldehyde does not interfere with these determinations. Formaldehyde gives some interference, particularly when the color is developed by heating (see Table I). The use of methanol as solvent was thus unsatisfactory, because of the presence of formaldehyde as an impurity.

The possibility of interference from other aldehydes and ketones can be predicted from Table I.

Because this method has been used in this laboratory chiefly for the analysis of mixtures of essential oils, a number of compounds commonly occurring therein were tested for interference. Such compounds as α-pinene, limonene, cadinene, eugenol, menthol, salol, linalool, methyl salicylate, salicylic acid, carvone, and phenol were without effect when present in reasonable amounts during the determinations of citral and cinnamaldehyde.

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Molybdenum Blue Reaction

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Areas of decreased density producing dips in the density-concentration curves made with molybdenum blue may be found and are reproducible about 50% of the time. Such dips take place at all points of the molybdenum blue spectra between 400 and 800 mμ; they are not associated with color changes. Under the conditions of these experiments the dips appear above 0.14 mg. of phosphorus (in 100.0 ml. of solution), but are not associated with specific changes in redox potential. The dip phenomenon represents a possible source of error when the molybdenum blue reaction is used for the determination of phosphorus with chlorostannous acid as the reducing reagent.

THE molybdenum blue reaction has been used for routine colorimetric determinations of phosphorus, molybdenum, silicon, and other constituents in a wide variety of materials. However, in spite of its importance, the mechanism of the reaction and the structure of molybdenum blue are not well understood. The equilibrium characteristics of the reaction (*2*) have been studied and the colloidal character of the molybdenum blue (*6*) has been established. The average valence of molybdenum in molybdenum blue appears to be about 5.67 and if reduction is carried further the density of the color decreases (*8*) and may change from blue to green or brown (*4*). Lesser color variations can be produced by varying other conditions (*5*).

Woods and Mellon (*9*), using sodium sulfite as a reducing reagent, reported that the blue color of molybdenum blue when used for the determination of phosphorus follows Beer's law up to 1.0 p.p.m. of phosphorus, above which a negative deviation takes place. Clausen (*1*), using chlorostannous acid as a reducing reagent, reported that the density-concentration curve obtained when the molybdenum blue reaction is used for the determination of phosphorus is not a straight line but a curve and that at a point above 0.14 mg. of phosphorus (in 100.0 ml. of solution) an area of decreased density may take place, producing a dip in the curve. This phenomenon is almost without parallel, although Lundberg (*3*) has observed a similar behavior when 2,6-dichlorobenzenone-indophenol is reduced with various reducing agents.

The present investigation was undertaken to ascertain whether or not the dip phenomenon is reproducible and is associated with a change in color or a decrease in density of a fixed color, and to attempt to correlate the dip phenomenon with changes in redox potential and pH.

EXPERIMENTAL

In the first series of experiments a number of density-concentration curves were made under varying conditions in an attempt to demonstrate that the dip phenomenon actually existed.

The method used was that of Todd and Sanford (*7*) with chlorostannous acid as the reducing agent for the determination of phosphorus in blood filtrates. Amounts of from 0.02 to 0.22 mg. of phosphorus, as potassium dihydrogen phosphate, in 6.0 ml. of 9% aqueous trichloroacetic acid solution, were used for the curves. To each of these were added 2.0 ml. of a reagent composed of equal amounts of 7.5% sodium molybdate and 10 *N* sulfuric acid and 2.0 ml. of a reagent made by dilution of a 60% solution of stannous chloride in concentrated hydrochloric acid to 400 volumes with water. The blue color produced is stable after about 5 minutes, at which time the solution is diluted to 100.0 ml. with water.

A large number of curves were obtained by three workers in two widely separated laboratories, using several batches of fresh reagents. All curves produced were nonlinear and about half of them exhibited the dip phenomenon; these results were independent of the worker or the laboratory. In every case the dips took place at the same place on the density-concentration curve. Simultaneously with the density determinations on some of the curves, the pH and redox potentials of the solutions were obtained to ascertain whether or not the dips were associated with changes in redox potential. The pH determinations were made in the event pH variations might require that the redox potentials be corrected by the amount of such variation. No such corrections were necessary. Figure 1 presents a set of typical data. The dip in the density-concentration curve over the range 0.14 to 0.18 mg. of phosphorus is clearly seen. The concomitant changes in the pH and redox potential curves are considered meaningless because they varied widely in shape from experiment to experiment. The pH and redox potential determinations were done on a laboratory model Beckman pH meter. The density data were obtained for the most part from a Coleman Universal spectrophotometer and partly from a Leitz photoelectric colorimeter.

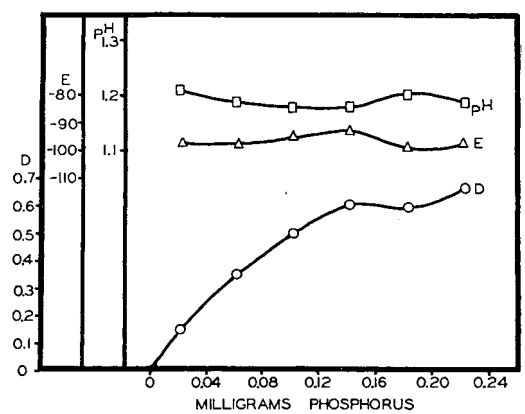

Figure 1. Inflection Point or Dip in Molybdenum Blue Color of Density-Concentration Curve from Phosphorus Determination

Changes in pH and redox potential are not significant. All points represent volume of 100 ml.

To ascertain whether or not the dip phenomenon existed only at the absorption maximum of molybdenum blue, absorption curves were obtained from a series of solutions of molybdenum blue made from varying amounts of phosphorus (Figure 2). The color was allowed to stand for 5.0 minutes before the density determinations were made. The proximity of the curve representing 0.14 mg. of phosphorus to that representing 0.18 mg. of phosphorus clearly indicates that the dip phenomenon exists over all parts of the absorption curve. The points at 710

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mμ (the absorption maximum) from each curve were plotted as a density-concentration curve on the same graph, using the concentration abscissas at the top. The resulting dotted line again shows the dip phenomenon.

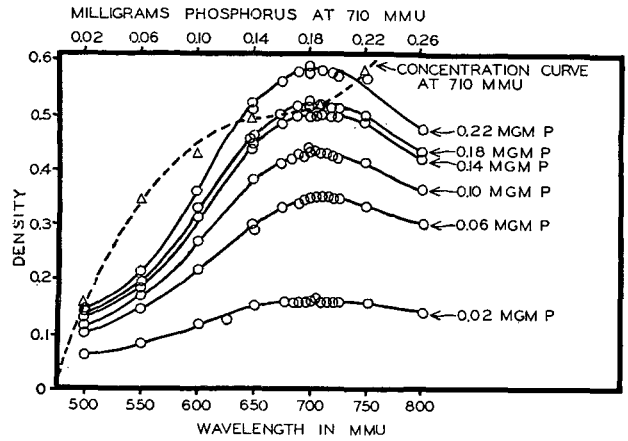

Figure 2. Absorption Curves of Molybdenum Blue Color from Phosphorus Determination Showing Dip Phenomenon

Dotted line represents points at 710 mμ from each curve plotted against concentration abscissas at top

Because it is necessary to allow solutions of molybdenum blue to stand for a few minutes for color development, it was of interest to ascertain the nature of this development and to trace the path followed by the redox potential during this period. Accordingly, samples containing 0.12 mg. of phosphorus each in 6.0 ml. of trichloroacetic acid were treated with reagents and allowed to stand varying lengths of time from 0 to 190 hours. They were then diluted to 100.0 ml. with distilled water as usual and pH, redox potential, and density were obtained. Typical data show the density dropping from 0.45 (the figure after the first 5 minutes) to 0.36, the redox potential rising from −90 to −67 mv., and the pH remaining approximately constant after 190 hours. No explanation for the rise in E is apparent. Figure 3 shows curves of this type extended from 0 to 16 minutes. As the redox potential becomes more negative—i.e., the molybdenum becomes more reduced—the density drops until, after 3 minutes, both curves level out. These factors suggest that the molybdenum is reduced by degrees and that in early stages the molybdenum blue molecule contains more vibrational energy than in subsequent stages. This explanation receives additional support

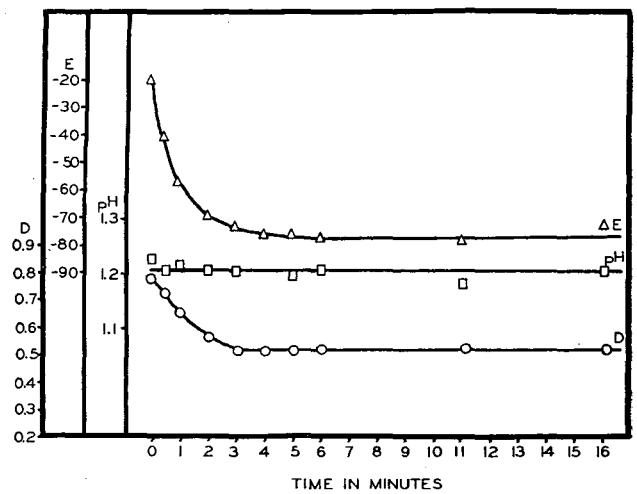

Figure 3. Development of Molybdenum Blue Color

Relation between density and redox potential as color develops

from the fact that the phosphomolybdic complex before reduction to molybdenum blue absorbs strongly in the near ultraviolet region.

ACKNOWLEDGMENT

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Chemical Analysis of Refinery C_5 Hydrocarbon Fractions

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PETROLEUM refinery distillate boiling between 4° and 51° C. (39° and 124° F.) at normal pressure is generally designated as the C_5 fraction. Hydrocarbons of most common occurrence in this range are listed in Table I. The number of these which make up a C_5 fraction varies widely, from only two, isopentane and *n*-pentane, in certain natural gasolines, to essentially all in naphtha from cracking operations conducted at 870° C. (1600° F.)

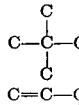
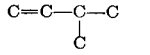
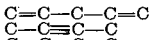
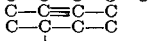
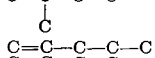
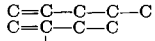
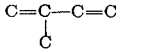
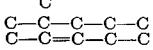
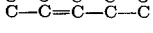
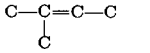
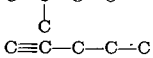
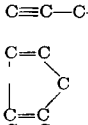
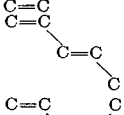
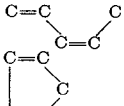
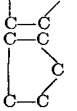
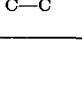
Recent developments have made the petroleum industry increasingly aware of the value of C_5 hydrocarbons as chemical raw materials. 1- and 2-pentenes comprise the starting materials in the production of *sec*-amyl alcohol, and thence methyl *n*-propyl ketone and *sec*-amyl acetate, all important as solvents. Tertiary C_5 mono-olefins are employed in the synthesis of *tert*-amylphenol, from which important chemicals and resins are manufactured. Isoprene is consumed in the formation of Butyl rubber and other polymeric substances. Cyclopentadiene and piperylenes also have valuable chemical and plastic derivatives. During the war a welcome supplement of high-grade motor fuel was obtained by feeding isopentane and C_5 mono-olefins to alkylation units. All these processes have required adequate hydrocarbon analytical methods in development and control.

After precise blending of the C_5 (pentene cut) vapors with a suitable diluent gas it is possible to employ established rapid and accurate gas analytical methods for the determination of conjugated diolefins, tertiary mono-olefins, total unsaturated hydrocarbons, and total saturated hydrocarbons. Detailed consideration is given the analysis and stability of samples containing the individual diolefins, isoprene, cyclopentadiene, and *cis*- and *trans*-piperylenes. A new colorimetric method specific for isoprene employing mercuric acetate reagent is described. By applying most of the above methods in an organized scheme to predetermined subfractions obtained in efficient distillation it is possible to define the composition of cracked fractions in terms of individual major components: neopentane, isopropylethylene, isopentane, 1-pentene, 2-methyl-1-butene, isoprene, *n*-pentane, 2-pentenes, trimethylethylene, cyclopentadiene, *trans*-piperylene, *cis*-piperylene, cyclopentene, and cyclopentane.

The following chemical procedures, which have been employed in these laboratories for the past several years, are designed to provide information on the composition of C_5 fractions even to the extent of practically complete definition in terms of individual components. Moldavskiĭ and co-workers (*15*) have described, meanwhile, a scheme of analysis based on the use of bromine and hydrogen bromide as selective reagents. This method, however, is difficult and applicable only to fractions free from diolefins, acetylenes, and naphthenes. Recently, the rudiments of mass and combination infrared and ultraviolet spectrometric methods have been developed (*2*, *23*), but these have not been found applicable to many samples without prior chemical treatment or distillation essentially by the techniques described below. In addition, the spectral methods require frequent calibration with expensive pure standard hydrocarbons representative of each component.

In the literature, a number of procedures employing chemical reagents are to be found which are claimed to be applicable to the estimation of the concentrations of conjugated diolefins, of tertiary mono-olefins, and of total unsaturated hydrocarbons in C_5 fractions. These would be difficult to integrate into a working scheme for a more or less complete analysis of the fraction because some require liquid samples; others, vapor. Those employing liquid samples generally provide the residues obtained after chemical treatment in such an uncertain state of composition as to be not particularly suited to further quantitative study. Analysis in the vapor state, on the other hand, obviates the objections to weighing and handling highly volatile liquids. Moreover, advantage can be taken of the rapid and accurate chemical techniques developed in recent years for the analysis of normally gaseous hydrocarbon mixtures.

Table I. Major Hydrocarbon Constituents of C_5 Fractions

Geneva Name	Common Name	Skeleton Structure	Type	Normal Boiling Point, ° C.
2,2-Dimethylpropane	Neopentane		Saturated hydrocarbon	9.45
3-Methyl-1-butene	Isopropylethylene		Branched primary mono-olefin	20.2
*****				
1,4-Pentadiene	.....		Nonconjugated diolefin	26.05
2-Butyne	Dimethylacetylene		β-Acetylene	26.9
2-Methylbutane	Isopentane		Saturated hydrocarbon	27.89
1-Pentene	*n*-Amylene		Primary mono-olefin	30.1
2-Methyl-1-butene	*unsym*-Methylethylethylene		Tertiary mono-olefin	31.05
2-Methyl-1,3-butadiene	Isoprene		Conjugated diolefin	34.07
*****				
Pentane	*n*-Pentane		Saturated hydrocarbon	36.0
2-Pentenes (*cis*- and *trans*-)	*n*-Amylenes		Secondary mono-olefins	36.4
2-Methyl-2-butene	Trimethylethylene		Tertiary mono-olefin	38.49
1-Pentyne	*n*-Propylacetylene		α-Acetylene	40.2
*****				
1,3-Cyclopentadiene	.....		Conjugated diolefin	41.0
trans-1,3-Pentadiene	*trans*-Piperylene		Conjugated diolefin	41.5
cis-1,3-Pentadiene	*cis*-Piperylene		Conjugated diolefin	43.8
Cyclopentene	.....		Mono-olefin	44.1
Cyclopentane	.....		Saturated hydrocarbon	49.20

VAPORIZATION AND BLENDING OF SAMPLE WITH DILUENT GAS

In choosing operation in the vapor phase two alternative techniques are available. The fraction may be maintained in the vapor state by (1) elevating the temperature, or (2) blending the vapor at room temperature with a gas, such as hydrogen, in order to prevent subsequent condensation. In the first case the analytical apparatus must be enclosed in a constant-temperature bath held about 80° C. or higher and thus manipulation becomes difficult. Moreover, use of acid absorption reagents at the higher temperature is open to question. The use of a blend of the vapor at ordinary temperatures has at least two outstanding advantages: (1) No further blending is required with samples that normally would be completely dissolved in an absorbing solution; and (2) the presence of diluent gas decreases the physical solubility of residual vapor in the reagents.

Various techniques are possible in blending the vapor of C_5 fraction. Primarily, of course, the sample should be free of all higher and lower boiling substances. This generally can be assured by distillation between 15° and 51° C. under normal pressure unless neopentane is present, when a lower limit of about 4° C. is required. Some C_4 hydrocarbons, such as ethyl- and vinylacetylenes, and 1,2-butadiene might be expected to be included at the lower temperature. Thermodynamic equilibrium data indicate, however, that these hydrocarbons and neopentane are not likely to be found together in any significant concentration in refinery samples from a given operation. For the same reason, 3,3-dimethyl-1-butene, boiling at 41.2°, and 2,2-dimethylbutane, 49.7°, are of very unusual occurrence (*7*).

The blending technique chosen and the gas law corrections involved have been described by Robey and Morrell (*19*).

Briefly, the liquid hydrocarbon, essentially free of acid gases and entrained water, is vaporized into a previously evacuated glass bulb of about 500-ml. capacity at a constant temperature not below 20°, until an absolute pressure of about 250 mm. is attained; hydrogen or other suitable gas is then admitted slowly until the total pressure rises to 750 mm. The blend is displaced from the bulb by mercury into analysis apparatus, as required, and is otherwise handled as a normal gas. The operation is readily adapted to the Podbielniak distillation apparatus (*17*) in which C_5 fractions are collected under reduced pressure in larger vessels and are displaced by a Töppler pump. Hydrogen was chosen as the diluent because it is readily available as a dry gas and generally involves no interference in subsequent analysis, although nitrogen and argon can also be employed under certain conditions.

To obtain the true mole fraction of hydrocarbon in the blend from the pressure readings it is necessary to take into account deviation from the gas laws. From approximate data (*19*) on pentane-hydrogen mixtures the compressibility of C_5 hydrocarbon vapor is about 1.024 at 250 mm. and that of a 33% mixture of C_5 in hydrogen, 1.011. Thus the mole fraction of hydrocarbon in the blend is actually $\frac{250 \times 1.024}{750 \times 1.011} = 0.338$, or an additive correction of +0.5 mole % is involved. It has been found convenient in this laboratory to prepare all blends of C_5 hydrocarbons at the above pressures and to use a single correction factor for the composition. However, lower hydrocarbon partial pressures can be employed and corrected accordingly. Higher partial pressures are likely to result in condensation of the hydrocarbon. Somewhat more rapid mixing is obtained if the diluent gas is introduced into the lower rather than the upper part of the blending vessel. Grease of the hydrocarbon-insoluble type should be used to lubricate stopcocks and joints in all apparatus containing these blends; and contact of the blend with hydrocarbon-absorbing rubber tubing should be held at a minimum. Mercury is preferable as the confining liquid in the blending and subsequent analysis because solubility effects are minimized and the analysis is kept on a dry basis.

CONJUGATED DIOLEFINS

The conjugated pentadienes, isoprene, cyclopentadiene, and the piperylenes (*cis-* and *trans-*) interfere with the subsequent determination of the tertiary olefins employing acid absorbents. They should therefore be removed or determined by absorption prior to further analysis. With the exception of *cis*-piperylene, this can be done by passing the dry blend in contact with a relatively small volume of molten maleic anhydride held at 100° in a manner practically identical to that used for the determination of butadiene (*9, 24*) in gases. Because maleic acid tends to polymerize the tertiary mono-olefins, it is necessary to employ maleic anhydride which is pure (melting point not lower than 52°), or which has been purified by redistillation in vacuo under the hood, and to which has been added about 1% of di-*n*-amylamine for purposes of deacidification as originally reported by this laboratory (*20*). In storage it is only necessary to exclude moisture from the purified maleic anhydride.

cis-Piperylene has been shown (*21*) to react only very slowly with maleic anhydride under the conditions just set forth. The *trans*-isomer reacts rapidly and quantitatively. This provides the key to the analysis of piperylene for its geometrical isomers. At higher temperatures and vapor concentrations, or when the sample is held under pressure in a liquid phase where peroxides may be present, the reaction is much less selective between the isomers (*3*). Conditions for best selectivity have been attained by bringing the blend into contact with maleic anhydride held at 56° (boiling acetone).

Because *cis*-piperylene is not readily absorbed by maleic anhydride, samples containing appreciable amounts of this isomer do not furnish a residue suitable for the subsequent analysis for tertiary mono-olefins. Fortunately, the *cis*-isomer is not present in very high concentrations in piperylenes derived from cracking operations; it amounts to only 20% of the piperylenes in C_5 fractions produced from gas oil at 870° C. (1600° F.), for example (*21*). Moreover, the piperylenes are removed, in most cases, from trimethylethylene, the closest boiling tertiary mono-olefin, by superfractional distillation, as is pointed out below.

The concentration of *cis*-piperylene can be estimated from the total diolefin content, when the total diolefin content is obtained from the difference of two determinations of unsaturation described in greater detail below—namely, the unsaturation as indicated by the application of (1) a reagent that absorbs completely all unsaturated components of the hydrocarbon-hydrogen blend, and (2) a catalyst that produces a volume contraction as the result of addition of hydrogen to each double bond (*4*). The absorption gives the sum of the mono-olefin and diolefin contents, and hydrogenation gives the mono-olefin plus twice the diolefin content. The difference between the two is the total diolefin content. The disparity between the values for diolefin content as determined by this method and by maleic anhydride absorption should closely approximate the *cis*-piperylene content. The main disadvantage of the determination by difference is the accumulation of the errors of the individual determinations. The nonconjugated diolefins and acetylenes which might be accounted for as *cis*-piperylene in this analysis, have not been found in significant concentrations in C_5 fractions examined to date. Exceptions perhaps are low-boiling 1,4-pentadiene and dimethylacetylene, which are easily separable from piperylene-containing subfractions by sample distillation.

In all analyses that involve absorption of hydrocarbons from the vapor blend, correction of the final results is required for deviation from the gas laws if the greatest degree of accuracy is to be obtained. The concentration of C_5 vapor in the blend is depleted by absorption and the residual gas becomes more nearly perfect.

For example, in the case of a blend of hydrogen and pure isoprene in contact with molten maleic anhydride, one can calculate the result that would be obtained if no correction were made. A measured volume of 100.0 ml. of the blend would occupy as an ideal gas $100.0 \times 1.011 = 101.1$ ml. This blend would contain $0.338 \times 101.1 = 34.2$ ml. of C_5 vapor measured as an ideal gas, all of which is absorbed by the maleic anhydride, leaving 66.9 ml. of hydrogen on which no appreciable correction is required. Making the calculations as usual, one obtains:

$$\frac{100 - 66.9}{100} \times 100 = \frac{33.1}{0.338} = 98.0 \text{ mole \% isoprene}$$

Thus an additive correction of +2.0% is required to obtain the true value.

Using this type of calculation, the corrections have been derived for all percentages of absorption from blends containing 25 and 33% of C_5 vapor and are presented graphically in Figure 1.

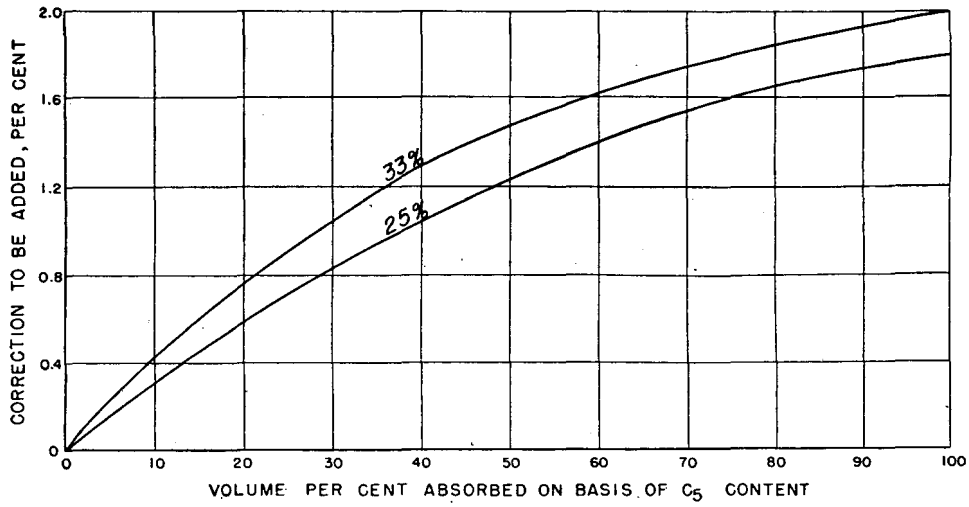


Figure 1. Correction Graph for Absorption of C_5 Hydrocarbons from 25 and 33 Mole % Blends with Hydrogen or Nitrogen

Table II. Analyses of Synthetic Mixtures Containing C_5 Diolefins and an Acetylene

Mole %	Component	Individual n_D^{20} [a]	Determination	Method	Mole Per Cent Based on Hydrocarbon in Blend: Synthesis	Found: Uncorrected [b]	Found: Corrected
100	Isoprene	1.4216	Reactive dienes [c]	Maleic anhydride	99+	96.8	98.8
37.0	Trimethylethylene	1.3871	Reactive dienes [c]	Maleic anhydride	57.8	56.2	57.8
22.8	*trans*-Piperylene	1.4311	Unsaturation	Hydrogenation	163.0	165.0	163.2
5.2	*cis*-Piperylene						
35.0	Isoprene	1.4217	.....	.....	...	...	...
100	Mixed piperylenes	1.4305	Unsaturates	87% H_2SO_4	100	97.6	99.6
46.4	*n*-Pentane	1.3577	Reactive dienes [c]	Maleic anhydride	29.3	28.1	29.1
16.5	Trimethylethylene	1.3869	Unsaturation	Hydrogenation	90.7	90.6	90.3
29.3	*trans*-Piperylene	1.4305	Unsaturates	87% H_2SO_4	53.6	52.8	54.4
7.8	*cis*-Piperylene		*cis*-Piperylene	Difference	7.8	9.7	7
100	Isoprene	1.4217	Unsaturation	Hydrogenation	200	202.0	199.6
100	*n*-Propylacetylene	1.3853	Unsaturation	Hydrogenation	200	202.6	200.2

[a] Best literature values for index of refraction: isoprene 1.4216; trimethylethylene 1.3873; mixed piperylenes 1.4309; *n*-pentane 1.3576; *n*-propylacetylene 1.385.
[b] Uncorrected for deviations from ideal gas behavior.
[c] C_5 dienes reactive to maleic anhydride under conditions taken as isoprene, cyclopentadiene, and *trans*-piperylene.

Table II presents data obtained upon the application of the gas analysis techniques and corrections outlined above to some synthetic mixtures of C_5 hydrocarbons. Mean deviation from synthesis of only about 0.3% is manifested by the corrected values. The *cis*-piperylene content obtained by difference of diolefin determinations by maleic anhydride and by unsaturation values is, of course, subject to a duplication of this error.

The generally assumed unreactivity of typical individual hydrocarbons other than conjugated diolefins with molten maleic anhydride is confirmed by the results in Table III. No anomalies are exhibited which would prevent the application of the reagent as outlined. The data do indicate, however, that physical solubilities in the reagent vary over a considerable range. It is evident that samples containing 2-pentenes and the acetylenes require the most care for complete physical saturation of the reagent prior to, and the maintenance of constant pressure during, the actual determination.

An effect which is not brought out by work on individual compounds is the increase of physical solubility resulting from the formation of the diolefin–maleic anhydride adduct in the course of the analysis. Error introduced by this effect should become evident in the analysis of mixtures containing conjugated diolefins. The fact that this is small in many cases is vouched for by the data in Table II. However, when highly soluble, but unreactive, hydrocarbons are present in the sample, the absorption may be carried out with fresh reagent followed by removal of the undissolved gases from the apparatus. A known volume of hydrogen or other inert gas may then be swept through the reagent, and that portion of the original sample which has been physically dissolved by the reagent determined by the increase in volume or by analysis of the sweeping gas.

Although the use of maleic anhydride as a reagent in purely chemical analysis of C_5 fractions has been emphasized in the above remarks, it has also been found feasible to employ this reagent, in the manner described, to remove reactive diolefins in order to reduce the complexity of mixtures submitted to analysis by infrared, ultraviolet, and mass spectrometric methods.

TERTIARY MONO-OLEFINS

Davis and Daugherty (*5*) and others (*16*) have demonstrated rather conclusively that 70% sulfuric acid may be applied as a selective reagent for the quantitative absorption of tertiary mono-olefins from a blend with diluent gas. Isopropylethylene, despite its branched structure, is functionally a primary olefin and hence does not interfere (*6*). The absorption pipet containing 70% sulfuric acid may conveniently be placed in connection with that for maleic anhydride described above. The experience of the authors has indicated the desirability of the use of a contact type of absorption pipet rather than the bubbler type and of the application of rational corrections for the coabsorption of other hydrocarbons calculated from residual absorption during uniformly timed periods of contact. The acid absorbent must, of course, be carefully saturated with nonreactive gas and corrections applied to the final results in accordance with Figure 1. In the use of Figure 1, the correction on the total of two absorptions in succession may be obtained from the graph just as for any single absorption. The correction on the second absorption is obtained by difference.

Table III. Behavior of Vaporized Hydrocarbons Found in C_5 Fractions toward Molten Maleic Anhydride at 100° C.

Compound	Solubility, Ml./2.5 Ml. [a]	Apparent Diolefin Content [b]
Isopropylethylene	1.3	0.0
1,4-Pentadiene	2.9	0.0
Dimethylacetylene	8.0	0.0
Isopentane	1.4	0.0
2-Methyl-1-butene	3.0	0.0
2-Pentenes (mixed)	7.3	0.0
n-Pentane	1.4	0.0
n-Propylacetylene	5.4	0.3
Cyclopentene	...	0.0

[a] Figures represent volume decrease after repeated contact of 100 ml. of 0.338 mole % blend of hydrocarbon in hydrogen gas at 1 atmosphere total pressure with 2.5 ± 0.5 ml. of anhydride inhibited with amine. Anhydride had not had previous contact with hydrocarbons.
[b] Expression of absorption from a subsequent blend of hydrocarbon with hydrogen after absorbent has been physically saturated as in solubility determination.

Cyclopentene is absorbed by 70% acid at a rate only somewhat slower than that of a tertiary mono-olefin. Cyclopentene would therefore be expected to be determined as one of the latter class.

The tertiary pentenes combine readily with anhydrous hydrogen chloride at temperatures as low as −80° C. (*12*). An attempt was made to adapt the apparatus of McMillan (*13*) to the determination, but only variable success was obtained even at elevated temperatures and with the use of argon and methane as condensable blending gases. Principal difficulties appeared to be the condensation of the amyl chloride product (boiling point 86°) and the rather large gas law corrections involved.

TOTAL UNSATURATED HYDROCARBONS

Two general gas analytical methods for determining the total concentration of unsaturated hydrocarbons—i.e., all mono-olefins, diolefins, and acetylenes—are widely employed at present. In principle one is based on volumetric absorption in a liquid reagent, the other on quantitative catalytic hydrogenation. Something of the efficacy and applicability of these methods has already been pointed out (Table II). Under suitable conditions the saturated hydrocarbons, isopentane, *n*-pentane, and cyclopentane, are not attacked and determination by difference is generally and acceptably accurate. If the percentages of conjugated diolefins and of tertiary mono-olefins are known, the sum of the concentrations of the normal mono-olefins and isopropylethylene can be calculated from the total unsaturation (provided only insignificant or known quantities of acetylenes and nonconjugated diolefins are present). Generally, the determination of total unsaturation is applied to a separate portion of blend or to a freshly prepared blend.

For an absorptive reagent, 87% sulfuric acid employed in a pipet in small portions in the manner described by Matuszak (*14*) has been found satisfactory. Among the many absorbent solutions described in the literature, those containing mercuric or silver salts or both have also given good results. Solutions that are highly aqueous contaminate the residual gas with water vapor, for which a correction is required. Solutions containing mercuric nitrate absorb hydrogen, necessitating preparation of the blend with nitrogen rather than with hydrogen. In either case, the corrections in Figure 1 apply.

The procedure and corrections involved in hydrogenation have already been reported (*19*). Briefly, equal volumes of additional hydrogen and of hydrogen-hydrocarbon blend are measured out and passed over an active catalyst at ordinary temperatures, and the contraction of volume is measured. Hydrogen essentially free of oxygen and moisture is required throughout. Blends prepared with nitrogen should not be employed because of the possibility of formation of ammonia. The apparatus for both unsaturation determinations may be integrated, if desired, with those described above for diolefin and tertiary olefin analysis.

The total unsaturation determinations may also be employed in conjunction with the infrared and mass spectrometric analysis. Spectral analysis of the residual gas from absorption or hydrogenation will provide, for example, further information on the concentration of branched paraffins and unsaturates. An alternative method of attaining the same end without the use of these instruments is set forth below.

ACETYLENES

It is generally known that α-acetylenes, which have the general formula RCH≡CH, tend to react with silver salts to form acetylides. Analytical procedures based on this reaction have been so well worked out that little additional detail can be justified here (*1, 26*).

Dimethylacetylene, having no active hydrogen, does not form a silver compound and must be determined otherwise. A method of accomplishing this, which has been disclosed recently (*26*), involves conversion to a ketal. It is also possible, because of significantly lower molecular weight, to isolate and concentrate dimethylacetylene in a lower-boiling subfraction, as explained below, and make the determination from vapor density measurements. The approximate composition of the subfraction must be otherwise known.

INDIVIDUAL DIOLEFINS

Generally speaking, up to this point consideration has been given only to determination of the various types of hydrocarbons, and indeed this extent of analysis suffices for many purposes. To resolve a cracked C_5 fraction further into individual components is a somewhat more lengthy but not especially difficult task.

It is evident from Table I that the boiling points of the individual members of the tertiary mono-olefinic, normal (primary and secondary) mono-olefinic, and paraffinic types are sufficiently removed from one another to make possible fairly complete separation by superfractional distillation. However, this is obviously not the case with the three diolefins, cyclopentadiene and the two piperylenes. Moreover, experience has shown that isoprene and the piperylenes, and isoprene and 1,4-pentadiene, are difficult to separate sharply by distillation when diluted by large amounts of paraffinic hydrocarbons. This is apparently the result of the deviations from Raoult's law even to the extent of azeotrope formation. Fortunately, a specific method for the quantitative estimation of cyclopentadiene has become available recently. In addition, the authors have found a set of conditions under which mercuric acetate produces a colored product as the result of reaction with isoprene. This reaction has proved to be adaptable toquantitative colorimetry and to be free of interference from other hydrocarbons likely to be present in C_5 fractions.

Table IV. Parameters for Rate of Dimerization of Lower Conjugated Diolefins in Liquid Phase[a]

Diolefin	*E*, Kg.-cal.	*A*	Reference
Butadiene	23	3.3×10^7	(*22*)
Isoprene	25.9	1.7×10^8	(*18, 27*)
Cyclopentadiene	16.4	3.6×10^9	(*10*)
trans-Piperylene	23	3×10^7	(*11*)
cis-Piperylene	?	Low	(*8*)

[a] Reaction: 2 diolefin ⟶ dimer.
Rate (gram moles per liter per hour) = A[diolefin]^2 exp. $(-E/RT)$

Stability of C_5 Conjugated Diolefins. No determination of C_5 conjugated diolefins is on a sound basis until consideration has been given the possibility of loss of these compounds from the fraction as the result of polymerization reactions. The polymers formed either prior to or during analysis are generally not detected by the methods applied for the determination of the monomers, and the polymers may probably be discarded in distillation without an accounting of their source.

The stability of butadiene has been studied by the authors (*22*), and further studies (*18*) indicate the behavior of the C_5 diolefins to be essentially analogous: Two major independent polymerization reactions can occur: (1) dimerization which is bimolecular and homogeneous in the liquid phase, and (2) polymerization to plastic substances of high molecular weight by a peroxide-catalyzed mechanism. The latter reaction is inhibited by the presence of an effective antioxidant and by the exclusion of air and other active oxygen-producing substances from the sample. For safety reasons, peroxides should not be permitted to accumulate in residua from distillation or evaporation to a point where explosive autoxidation may take place. Rapid peroxidation and explosion have been experienced at the end of batch distillation of C_5 diolefins when air is siphoned into the warm still pot.

Neither conventional inhibitors nor peroxides have any effect on the rate of the dimerization reaction. The amounts of the various dimers formed are therefore governed for all practical

purposes by the thermal history of the sample. Although dicyclopentadiene in the sample can be reconverted quantitatively to the monomer for subsequent determination (*25*), conditions have not yet been found whereby this can be accomplished with the dimers of isoprene and the piperylenes. Consequently, nothing can replace a knowledge of the history of the sample, together with storage at low temperatures and for short periods of time prior to analysis.

The rates of dimerization of the individual C_5 conjugated diolefins are given in Table IV. From these it can be calculated that the rate of dimerization for cyclopentadiene at ordinary temperatures is several orders of magnitude faster than for the other lower diolefins. In fact, under conditions conducive to poor heat removal, there is always danger that in the case of pure cyclopentadiene the reaction may become violently autogenous. The rate in the case of *trans*-piperylene is about the same as for butadiene, while that of isoprene is slower at ordinary temperatures, but faster at temperatures above 140° C. It is reported that *cis*-piperylene exhibits an anomalously slow rate of dimerization and this is thought to be related to its reluctance to undergo Diels-Alder condensation, already known to be manifest in the case with maleic anhydride. Experience has indicated that codimerization of cyclopentadiene with the various aliphatic diolefins is not a significant factor.

Cyclopentadiene. Uhrig *et al.* (*25*) have shown recently that cyclopentadiene can be determined colorimetrically with a minimum of interference from other hydrocarbons in the fraction. The very active methylene group condenses with benzaldehyde in the presence of alcoholic potassium hydroxide to produce a stable, colored fulvene derivative. Ward's method, involving condensation with acetone instead of benzaldehyde, has been employed in this laboratory successfully. Antioxidants should be absent from the portion of the sample tested.

Isoprene. This method depends upon the fact that isoprene forms a yellow color with solutions of mercuric acetate in absolute methanol. The optical densities of the cooled and filtered solutions are determined spectrophotometrically. The amount of isoprene in the unknown is calculated from that in a known by means of Beer's law. In general, the present procedure is applicable to C_5 fractions containing from 5 to 50% isoprene and the concentration of the piperylenes should not be more than twice that of isoprene. Freshly distilled samples are required, free of peroxides and sulfur compounds; small amounts of antioxidants can be tolerated.

Place about 50 ml. of absolute methanol in a clean dry 100-ml. glass-stoppered volumetric flask and weigh to the nearest milligram. Pipet exactly 1 ml. of cold, dry sample into the flask, keeping the tip of the pipet slightly below the surface of the methanol. Stopper the flask, shake it without permitting the stopper to become wet, and weigh again to the same tolerance. Make up the solution to 100 ml. with methanol and mix thoroughly.

A suitable standard solution of isoprene can be prepared by diluting exactly 1 ml. of C.P. isoprene (available from the Phillips Petroleum Company), or a suitable secondary isoprene of known purity, to 100 ml. with methanol as described above. Portions of standard solution may then be diluted further with methanol to provide a concentration of isoprene roughly equal to that in the sample to be analyzed. The standard solution is stable for several days in the refrigerator.

Weigh 2.5 grams of mercuric acetate (Mallinckrodt's has been found suitable; in any case, 1 gram should be completely soluble in 4 ml. of methanol at 60° C.) into each of several heavy-walled lipped Pyrex test tubes about 15 × 1.3 cm. (6 × 0.67 inches) inside diameter: two tubes for each sample, standard, and blank. Pipet 10 ml. of absolute methanol into each tube and exactly 1 ml. of diluted sample or standard into the designated tubes. The blanks are prepared by simply adding 1 ml. of methanol. Shortly thereafter stopper the tubes, secure the stoppers tightly (rubber should be heated with a separate portion of reagent prior to use), and place the tubes in a thermostatic liquid bath maintained at 60° ± 1° C. Agitate the tubes to dissolve the solid reagent rapidly and essentially completely, and permit them to remain in the bath for 60 minutes.

Remove the tubes, cool under the tap, and filter into individual absorption cells, first tapping the tube to break up any precipitated cake. Stopper the absorption tubes and proceed without delay to the spectrophotometric measurements. Determine the optical density of the filtrates as compared to pure methanol in a spectrophotometer or a colorimeter at an approximate wave length of 4300 Ångström units.

Employing this procedure the data in Table V have been obtained on synthetic mixtures comprising mainly C_5 hydrocarbons. The mean deviation observed with these was less than 3% based on isoprene. Butadiene shows no tendency to interfere.

Piperylenes. Knowing the isoprene and cyclopentadiene contents of a C_5

Table V. Isoprene Determination in Synthetic Mixtures by Mercuric Acetate Method[a]

Synthesis, Weight %		Isoprene Found, Weight %
31.0	Isoprene	29
69.0	Trimethylethylene	
20.0	Isoprene	20
20.0	*trans*-Piperylene	
60.0	Trimethylethylene	
15.0	Isoprene	15
72.0	Pentene-2	
13.0	Butadiene	
100.0	Trimethylethylene	0.3
4.9	Isoprene	4.9
95.1	Diolefin-free C_5 fraction from refinery steam cracking[b]	
10.6	Isoprene	11.1
89.4	Diolefin-free C_5 fraction as above	
20.3	Isoprene	21.1
79.7	Diolefin-free C_5 fraction as above	

[a] Pure C_5 compounds employed were of same quality as in Table II.
[b] Diolefins removed by reaction with maleic anhydride.

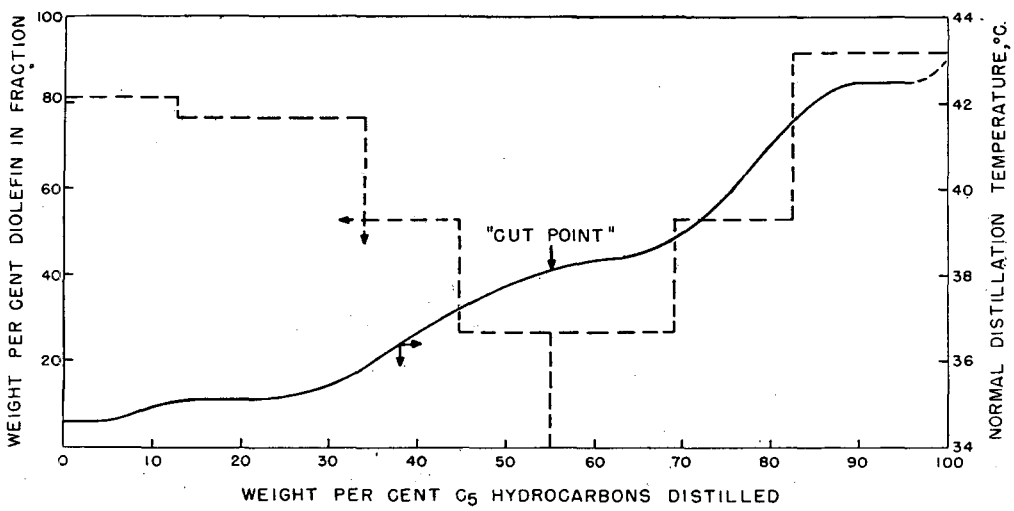


Figure 2. Analytical Distillation of Synthetic Mixture of Isoprene, Trimethylethylene, and Piperylenes

Table VI. Scheme for Complete Analysis of C_5 Fractions

Subfraction	Boiling Range, ° C.		Determination or Calculation	Component
I	4.3[a] to 24		Total unsaturates by absorption	Isopropylethylene
			Remainder	Neopentane
II	24 to 33.5	A.	Total unsaturates by absorption	
		B.	Diolefins reactive with maleic anhydride	Isoprene in this cut
		C.	Tertiary mono-olefins in residual vapor from B	2-Methyl-1-butene
			A − (B + C)	1-Pentene[b]
			Remainder	Isopentane
III	33.5 to 41.3[c]	C.	Total unsaturates by absorption	
		D.	Diolefins reactive with maleic anhydride	
		E.	Tertiary mono-olefins in residual vapor from B	2-Methyl-2-butene
			C − (D + E)	2-Pentenes[c]
			Remainder	*n*-Pentane
IV	41.3 to 51		Total unsaturates by absorption	Cyclopentene[e]
			Remainder	Cyclopentane
Original C_5 fraction or any of above subfractions as required		F.	Total unsaturates by absorption	
		G.	Diolefins reactive with maleic anhydride[d]	
		H.	Cyclopentadiene by fulvene reaction	Cyclopentadiene
		I.	Isoprene by mercuric acetate reaction	Isoprene[d]
		J.	Total acetylenes by ketal reaction	
		K.	α-Acetylenes by $AgNO_3$ reaction	Propylacetylene
		L.	Total unsatn. by hydrogenation	
			J − K	Dimethylacetylene
			G − (H + I)	*trans*-Piperylene
			L − (F + J + G)	*cis*-Piperylene and/or 1,4-pentadiene

[a] With highly cracked samples an initial temperature of 15° is recommended.
[b] This value should be corrected for any 1,4-pentadiene and dimethylacetylene present as determined by additional analysis.
[c] This value should be corrected for any isoprene, cyclopentadiene, acetylenes, *trans*-piperylene, or *cis*-piperylene present as determined by additional analysis.
[d] When concentration of diolefins is greater than 50% an extra subfraction should be obtained by making a cut at 38.2°.
[e] This value should be corrected for any cyclopentadiene, acetylenes, *trans*-piperylene, and *cis*-piperylene present as determined by additional analysis.

fraction by application of the procedures outlined just above, the authors determined the concentration of piperylenes from the total diolefin content by difference within the limitations already set forth.

When the concentration of conjugated diolefins is greater than 50%, sufficient separation of isoprene and the piperylenes for analytical purposes can be accomplished by fractional distillation. This is illustrated by the data plotted in Figure 2, which represent results obtained on distillation of a synthetic mixture of pure isoprene, trimethylethylene, and piperylenes. The column employed was rated at 50 theoretical plates under total reflux. A reflux ratio of 20 was actually employed. Figure 2 gives the recorded distillation temperatures under atmospheric pressure and the diolefin contents of various subfractions segregated in the course of the experiment. Benzene was added to the initial mixture to displace the C_5 hydrocarbons from the column. When all of the diolefin distilling below the mid-boiling temperature, 38.2°, is accounted as isoprene and that above as piperylenes, the following weight per cent figures result:

	Synthesis	Found
Isoprene	34.7	35.3
Piperylenes	27.6	27.6

These figures, of course, do not necessarily indicate that a perfect separation of the diolefins was obtained. On the contrary, there is evidence in Figure 2 that appreciable "overlapping" occurred. However, in the usual case when neither diolefin is present in large excess over the other, the resulting potential errors probably tend to compensate.

COMPLETE ANALYSIS BY AN INTEGRATED SCHEME

It has perhaps become evident that the complete analysis of C_5 fractions for major individual components might be accomplished by fractional distillation into subfractions and chemical analysis of these subfractions. A re-examination of Table I indicates that the hydrocarbons likely to be present have been divided into groups of close boiling substances separated by asterisk which might correspond to subfractions in distillation. Each group contains not more than one common hydrocarbon of a given type; exception is found in the fourth group, which contains three conjugated diolefins. In setting up these groups particular attention has been paid to obtaining the best probability of separation of the mono-olefins and of the saturated hydrocarbons by distillation. The separation of the individual diolefins is not so critical when the sum of the individual concentrations is less than 50% because of the availability of specific analytical methods, which can be applied to the subfractions or to the original C_5 fraction or both. At higher concentrations, isoprene and the piperylenes can be determined by the method involving distillation to the special "cut point" indicated above, followed by chemical analysis of the resulting fractions. These fractions can be obtained in the course of the distillation outlined below or upon distillation of a separate portion of sample.

The recommended normal boiling ranges of the fractions to be collected in the more general cases are shown in Table VI, together with an outline of the subsequent analyses necessary to obtain essentially complete breakdown. Higher and lower boiling substances should, of course, be collected and set aside for any disposition desired. A quantity of somewhat higher boiling material greater than the holdup of the column should be present to act as a "chaser" of the C_5 hydrocarbons. Addition of quantities of C.P. benzene, which has a negligible tendency to form azeotropes with highly cracked cuts, plus an antioxidant, or of hexane or heptane for others, will make up any deficiency in this regard.

The amount of sample to be distilled will vary with the degree of detail required in the final results. In any case the holdup of the column employed should be considered. From 20 to 50 times the volume held up during the distillation is generally the established minimum. A 60-ml. charge to the 90-cm. (36-inch) Hyd-Robot Heli-grid packed column (*17*), which is employed

Table VII. Analysis of Synthetic Mixtures of C_5 Hydrocarbons

(Mole per cent)

Component	Sample I Syn.	Sample I Found[a]	Sample I Found[b]	Sample II Syn.	Sample II Found[b]	Sample II Found[b]	Mean Deviation
Neopentane	...	...	...	1	...	2	1.0
Isopropylethylene	2	2	2	2	2	2	0.0
Isopentane	39	43	37	40	40	40	1.5
1-Pentene	9	9	10	12	13	14	1.0
2-Methyl-1-butene	9	9	8	9	9	8	0.5
n-Pentane	5	5	6	9	9	8	0.5
2-Pentenes	14	13	14	16	15	15	0.75
Trimethylethylene	21	18	21	9	9	10	1.0
Cyclopentene	...	...	...	1	1	0	0.5
Cyclopentane	1	1	2	1	2	1	0.5
Total	100	100	100	100	100	100	

[a] Hyper-cal column.
[b] Hyd-Robot column.

rather widely in the petroleum industry, has been found adequate for complete analysis. The 90 × 1.25 cm. (36 × 0.5 inch) Hyper-cal column (*17*) requires from 250 to 1000 ml. and should be operated with a reflux ratio of at least 25. Undoubtedly, any of a number of columns having equal or better efficiency can be used just as well, if means are provided for maintaining condenser temperatures at 0° and lower during most of the distillation period.

Employing the specific columns mentioned above, the results presented in Table VII were obtained on synthetic mixtures which simulate the composition of C_6 fraction from certain common types of cracking operations. These figures show that the mean deviation of the determined value from synthesis is somewhat less than 1% based on total sample. This deviation is in line with the error in vapor blend analysis cited earlier and the probable errors involved in the distillation step. Somewhat greater error, a total of probably about 1% based on total sample, would be expected when diolefins are present in significant amounts.

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GLUCOSE

A Direct Colorimetric Method for Determining Carbohydrates

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The molybdenum blue reaction is described as a quantitative method for the direct colorimetric determination of glucose. The method involves the formation of a heteropoly complex with molybdate and phosphate, and its subsequent reduction to molybdenum blue upon heating with reducing sugar. The intensity of color increases with heating time. The reaction time chosen gives the maximum sensitivity with a minimum of interference from reducing disaccharides. The method is suitable for the determination of glucose in the presence of moderate amounts of sucrose and other di- and trisaccharides. This principle may be extended to the determination of other sugars.

REDUCING agents in the presence of phosphates, silicates, or certain other acid radicals react with molybdates to produce the familiar substance known as molybdenum blue (*8*). Several observers have noted that reducing sugars react with ammonium molybdate to develop a blue color when heated and have utilized this reaction for qualitative and approximate quantitative tests for sugars (*1*). The conditions for a more sensitive colorimetric determination for reducing sugars based upon the molybdenum blue reaction have been established by Gilbert and Neapass (*3*) with ammonium molybdate and potassium dihydrogen phosphate. In an effort to apply this reaction to sugar analysis by means of the photoelectric colorimeter, the present authors have investigated these conditions (*3*) for glucose.

EARLIER APPLICATIONS

The molybdenum blue reaction has been used in the sugar industry to detect traces of sucrose by its reducing effect on molybdates in acid solution. Pinoff and Gude (*7*) found that fructose reacted much more quickly than aldoses, and that larger amounts of glucose or lactose were required to give the test than is the case with fructose. Dorfmüller (*2*) stated that ammonium molybdate may be substituted for α-naphthol to detect sugar in factory condensates and sweet waters.

The molybdenum blue reaction has been modified by Matthews (*6*), who made it applicable to approximate quantitative estimations of sucrose in samples such as entrainment waters from sugar pans which contain a negligible quantity of interfering

reducing substances. Matthews considered the test to be delicate and reliable and to give uniform results. Gilbert (*3*) has applied the reaction to determine sugars obtained from tung leaves and hydrolysis products of tung and potato starch.

A qualitative study of the color reaction with ammonium molybdate and a number of sugars was made in 1944 by Lo and Chu (*5*). These investigators obtained blue colors by heating 5% solutions of glucose, galactose, fructose, maltose, lactose, and sucrose after treating each sugar with 3 ml. of 1% ammonium molybdate and 1 ml. of 3 *N* sulfuric acid.

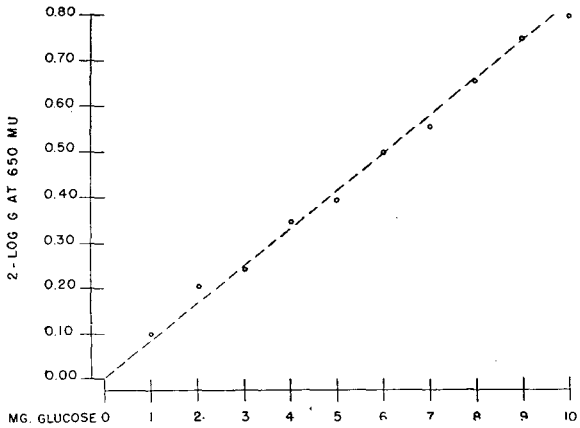

Figure 1. Standard Curve for Determination of Glucose by Molybdenum Blue Reaction

Quantitative application of the molybdenum blue method by reaction of a carbohydrate directly with molybdate has largely been limited to the approximate estimation of sucrose in sugar factory wastes and condenser waters. Available molybdate methods for sucrose require that the sugar be hydrolyzed by acid to form glucose and fructose, and are, thus, in the heating time specified, dependent upon the fructose present.

While the reaction obtained by Lo was positive for glucose, it was necessary to have up to a 5% solution of carbohydrate to develop the color upon heating at boiling temperature. However, the conditions set for the experiment by Gilbert are more favorable. He used 5 ml. of sugar solution containing 20 to 50 mg. of glucose, and obtained a blue color upon boiling for 20 to 30 minutes in a water bath. Although Gilbert increased the sensitivity appreciably, it was still far from the level of attainment possible for the molybdenum blue reaction for elements like tin, germanium, arsenic, or phosphorus, where the sensitivity is measured in parts per million. High sensitivity was also obtained by Heard and Sobel (*4*), who applied the reaction to the determination of reducing steroids. But, fundamentally, the molybdenum blue reaction for sugars showed possibilities, and it was undertaken in this project to develop the reaction as a quantitative colorimetric method for glucose.

EXPERIMENTAL

Reagents. Potassium dihydrogen phosphate, C.P., 0.02 *M*. Ammonium molybdate, C.P., 7.5%. Highest grade sugars.

Procedure. The reaction was carried out at the optimum pH of about 5.3 as follows: Into each of several 25-ml. volumetric flasks, 5 ml. of 0.02 *M* potassium dihydrogen phosphate and 10 ml. of 7.5% ammonium molybdate were introduced. Amounts of the sugar under study varying from 1.0 to 10 mg. were added and the flasks were made up to volume. After the solution had been mixed and the stoppers removed, the flasks were covered with a piece of sheet metal to prevent contamination from condensing steam, and heated in an autoclave, using open steam, at 100° C. for exactly 30 minutes. They were cooled at once in ice water to room temperature which stops the reaction. The color intensity was read in a Coleman Model 11 Universal spectrophotometer at a wave length of 650 mμ, using *19-mm. tubes*.

Precision. A standard curve for glucose was prepared and found to conform to the requirements of Beer's law (Figure 1). Thirteen identical determinations gave a standard deviation of 1.23 for galvanometer readings, with a mean value of 58.59.

Interference Due to Other Sugars. All monosaccharides yield a blue color under these conditions (Table I), but disaccharides and raffinose are affected to a very small extent. Only high concentrations of maltose cause appreciable interference. The intensity of color increases with increase in the heating time up to about 4 hours of heating at 100° C. for glucose. Results of numerous experiments pointed to a heating time of 30 minutes for the determination of glucose in the presence of di- and trisaccharides. Less heating time would reduce the sensitivity appreciably, while more heating time would increase interference from reducing disaccharides.

Results with glucose-sucrose mixtures using up to 10 mg. of glucose are given in Table II. It is evident that the presence of sucrose in glucose solutions does not appreciably affect the readings, unless the sucrose-glucose ratio and the total amount of sugar are both high. It is the authors' practice to use a concentration such that the aliquot to be analyzed contains between 2 and 5 mg. of glucose. When the sucrose-glucose ratio does not exceed 2 to 1, the results obtained are valid for the glucose content of the sample.

The amount of inversion by this procedure is almost negligible. When samples of hard candy manufactured from glucose and sucrose were assayed for glucose, results with the molybdenum blue reaction (23.5%) gave excellent agreement with those obtained by the Munson and Walker gravimetric method (23.8%).

DISCUSSION

This study treats of the application of the molybdenum blue reaction to the quantitative estimation of glucose in the presence of sucrose, and provides the nucleus for the further development of this test for determining other carbohydrates. Basically similar methods could be developed for the determination of any one of the reducing monosaccharides in the presence of disaccharides and other noninterfering substances. The possibility of applying this method to the determination of other carbohydrates like maltose, starch, and glycogen that are hydrolyzable to glucose is indicated. The further possibility of developing this method for the determination of the more reactive in the presence of the less reactive sugars is also suggested. This prob-

Table I. Transmittancy at 650 mμ Developed with 1 Mg. of Sugar in 30 Minutes' Heating Time Using 19-mm. Tubes

	Galvanometer Readings
Arabinose	44.5
Galactose	50.5
Levulose	61.5
Glucose	76.5
Xylose	71.5
Mannose	75.5
Maltose	95.8
Lactose	99.3
Sucrose	98.8
Raffinose	99.5

Table II. Transmittancy at 650 mμ of Increasing Quantities of Glucose in Mixtures of Glucose and Sucrose

(Galvanometer readings at 650 mμ using 19-mm. tubes for glucose-sucrose mixtures of percentage composition indicated)

Glucose, Mg.	100% G 0% S	80% G 20% S	60% G 40% S	40% G 60% S	20% G 80% S
0	100	100	100	100	100
2	66.6	63.2	64.2	62.3	64.7
4	47.0	44.3	45.9	42.4	45.8
6	33.5	31.0	32.6	33.7	30.2
8	26.8	26.6	26.4	25.8	22.2
10	21.0	18.0	17.3	17.7	18.2

lem could be solved by reducing the heating time to the point where the slower reacting monosaccharides are negative while the faster reacting monosaccharides are positive. Finally, by heating a mixture of sugars for various durations of time and measuring the subsequent color developed, binary and ternary mixtures of sugars might possibly be determined by mathematical analysis of the results obtained.

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Determination of Tetraethyl Pyrophosphate in Mixtures of Ethyl Phosphate Esters

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An analytical method for determining tetraethyl pyrophosphate in mixtures of ethyl phosphate esters involves selective hydrolysis of the higher polyphosphates and separation of tetraethyl pyrophosphate, triethyl phosphate, and a small amount of diethyl acid phosphate by benzene extraction. The diethyl ester is neutralized and the tetraethyl pyrophosphate is determined by hydrolysis with alkali. Triethyl phosphate does not interfere.

THE principal insecticidally active ingredient in mixtures of ethyl phosphates such as "hexaethyl tetraphosphate," technical tetraethyl pyrophosphate, etc., is generally recognized to be tetraethyl pyrophosphate:

$$(C_2H_5O)_2PO—O—PO(OC_2H_5)_2$$

Other constituents of commercial preparations are higher ethyl polyphosphates, ethyl metaphosphate, and triethyl phosphate. There is, therefore, a need for a simple analytical procedure for determining the tetraethyl pyrophosphate content of mixtures of ethyl phosphate esters.

Hall and Jacobson (*1*) recently suggested a procedure involving selective hydrolysis in water at 0° C., followed by chloroform extraction of the pyrophosphate and any triethyl phosphate present. The chloroform extract is distilled to give a mixture of the two esters, which is analyzed for pyrophosphate by determining the refractive index. The principal drawback of the method is that it is based on distillation of tetraethyl pyrophosphate, a thermally unstable material.

A simpler method, avoiding the difficulties of quantitative distillation of an unstable compound, involves selective hydrolysis in aqueous sodium chloride solution, under conditions carefully chosen to ensure complete hydrolysis of the higher polyphosphates and minimal (and predictable) hydrolysis of tetraethyl pyrophosphate. The hydrolysis solution then is extracted with benzene. The benzene extract, containing tetraethyl pyrophosphate, triethyl phosphate, and a small amount of diethyl acid phosphate, is treated with excess alkali after neutralization of the diethyl ester. This hydrolyzes the tetraethyl pyrophosphate without affecting the triethyl phosphate. The tetraethyl pyrophosphate content is calculated from the consumption of alkali, taking into account a small loss in aqueous salt solution.

APPARATUS

A dropper weighing bottle (5 ml.) with ground-glass joint, two pear-shaped 60-ml. separatory funnels, with stem cut off about 0.95 cm. (0.375 inch) below stopcock and ground at 45° angle, and a 250-ml. Erlenmeyer flask are used.

REAGENTS

Reagents include sodium chloride solution, containing 9 parts of sodium chloride per 100 parts of solution; nitration grade benzene; 0.5 *N* sodium hydroxide and 0.5 *N* hydrochloric acid, accurately standardized; and bromothymol blue indicator solution, 0.1% in water, solubilized as sodium salt.

PROCEDURE

Selective Hydrolysis. Place about 3 to 5 ml. of sample in a weighing bottle. Do not allow the material to wet the ground-glass portion of the weighing bottle and replace the dropper immediately to avoid moisture pickup. Weigh the bottle and sample to the nearest milligram. Place 20 ml. of 9% sodium chloride solution in a separatory funnel, marking this funnel A and a second funnel B. Adjust the temperature of the salt solution to 30° C. by running warm or cold water over the surface of the funnel.

Add approximately 1.0 to 1.5 grams (samples containing 50% tetraethyl pyrophosphate or more) to 2.0 to 2.5 grams (samples containing less than 50% tetraethyl pyrophosphate) of the sample to the salt solution in separatory funnel A, stopper, shake until the solution is homogeneous, and let stand exactly 5 minutes from the time the sample was added. Reweigh the weighing bottle to the nearest milligram to determine the exact sample weight.

Extraction. After 5 minutes, add 20 ml. of benzene to the solution in separatory funnel A and shake vigorously for 30 seconds. Allow the layers to separate and draw off the aqueous (lower) layer to separatory funnel B, taking care that none of the benzene enters the stopcock bore. Wash the benzene layer in A with 5 ml. of 9% sodium chloride by shaking for 10 seconds, separate the layers, and draw off the aqueous layer to funnel B, this time allowing the benzene interface to pass just through the stopcock bore.

Extract the combined aqueous layers in separatory funnel B with 10 ml. of benzene for 10 seconds, separate the layers, and draw off and discard the aqueous (lower) layer, keeping the benzene interface above the stopcock bore. Wash the benzene in B with 5 ml. of 9% sodium chloride by shaking for 10 seconds, separate the layers, and draw off and discard the aqueous layer, allowing the interface to pass just through the stopcock bore. Drain the benzene layer in separatory funnel B into funnel A.

Wash the combined benzene layers in funnel A with 20 ml. of ice-cold distilled water by shaking for 10 seconds. Separate the layers and drain the aqueous (lower) layer into a 250-ml. Erlenmeyer flask, keeping the interface above the stopcock bore. Add

5 to 10 drops of bromothymol blue indicator solution and neutralize immediately with 0.5 *N* sodium hydroxide to the bromothymol blue color change, taking the first definite blue as the end point.

Assay. Run the benzene layer in funnel A into the cold water wash previously neutralized in the Erlenmeyer flask. Rinse the walls of funnel B with 5 ml. of benzene, drain into funnel A, use this benzene to rinse the walls of funnel A, and drain into the Erlenmeyer flask.

Add to the benzene-water mixture in the Erlenmeyer flask approximately twice as much 0.5 *N* sodium hydroxide as is required for the expected amount of tetraethyl pyrophosphate (use 27 ml. of 0.5 *N* sodium hydroxide per gram of tetraethyl pyrophosphate expected), and stir or shake for 1 hour vigorously enough to obtain good mixing of the two phases. After 1 hour, titrate to the bromothymol blue end point with 0.5 *N* hydrochloric acid, swirling the flask to mix the benzene and water layers. The benzene layer will occlude droplets of water. It is, therefore, necessary to let the layers separate completely after each addition of standard acid or base when close to the end point. Because the first definite blue is taken as the end point, more accurate results are obtained by overtitrating with 0.5 *N* hydrochloric acid to a definite yellow and then titrating back to the blue end point with 0.5 *N* sodium hydroxide. The end point is sharp and stable. Record the net volume of 0.5 *N* sodium hydroxide consumed (not including the volume required for the neutralization of the water wash).

Calculation. Tests with pure tetraethyl pyrophosphate indicate that 97.8% of that present is recovered in the analysis. Of the remaining 2.2%, 1.3% is lost by hydrolysis during the 5-minute standing period before extraction, while 0.9% remains unextracted. This loss of 2.2% is taken into account in the following calculation:

$$\frac{\text{Net ml. of 0.5 } N \text{ NaOH} \times 7.255}{\text{wt. of sample} \times 0.978} = \% \text{ tetraethyl pyrophosphate}$$

or, simplifying,

$$\frac{\text{Net ml. of 0.5 } N \text{ NaOH} \times 7.42}{\text{wt. of sample}} = \% \text{ tetraethyl pyrophosphate}$$

DISCUSSION OF PROCEDURE

The conditions recommended for the preliminary hydrolysis are those found to be most favorable for the complete hydrolysis of the higher polyphosphates with minimal loss by hydrolysis of tetraethyl pyrophosphate. If the temperature of hydrolysis is reduced below the specified 30° C., the time for hydrolysis of the higher polyphosphates is increased. As the rate of hydrolysis of tetraethyl pyrophosphate is not reduced proportionately at lower temperatures, greater losses of the latter are incurred. For example, at 0° 2.5 hours are required for complete hydrolysis of the higher polyphosphates, during which 4.5% of the pyrophosphate is lost, whereas at 30° only 5 minutes are required, during which 1.3% of the tetraethyl pyrophosphate is hydrolyzed.

Sodium chloride solution is used instead of water for the preliminary hydrolysis to increase the extractive efficiency of benzene. Higher concentrations than the recommended 9% tend to increase extraction of diethyl acid phosphate.

Benzene is chosen rather than chloroform as the extracting solvent because it has less tendency to extract ethyl metaphosphate and acidic hydrolysis products, and because of its stability toward sodium hydroxide.

The benzene extract contains only tetraethyl pyrophosphate, triethyl phosphate, and diethyl acid phosphate. Most of the diethyl ester remains in the aqueous salt solution, together with the other acidic hydrolysis products and ethyl metaphosphate. Less than 1% of the tetraethyl pyrophosphate is unextracted.

Any diethyl acid phosphate present in the benzene solution is extracted by the cold water wash and then neutralized. Some tetraethyl pyrophosphate is also extracted, but its rate of hydrolysis in cold water is so low that only a negligible amount is hydrolyzed and titrated. The unhydrolyzed pyrophosphate in the water layer is not lost in the assay, as the water and benzene layers are recombined for the final hydrolysis.

Hydrolysis of tetraethyl pyrophosphate in benzene solution by stirring with excess sodium hydroxide at room temperature is more than 98% complete in 30 minutes and quantitative in 45 minutes to 1 hour. The hydrolysis product of tetraethyl pyrophosphate is diethyl acid phosphate, a strong acid which can be readily titrated alkalimetrically. The equivalence point coincides closely with the color change of bromothymol blue if the first definite blue color is taken as the end point.

Triethyl phosphate, which is hydrolyzed very slightly by aqueous sodium hydroxide at room temperature, is completely unattacked in the presence of benzene.

The precision of the method is ±0.5% of the tetraethyl pyrophosphate content, as shown in the duplicate analyses of Table I.

An individual determination requires about 1.25 hours, but the average time per analysis can be reduced without difficulty to 30 minutes or less by analyzing a number of samples concurrently.

Table I. Precision of Method

Sample	Tetraethyl Pyrophosphate, %	Deviation from Average, %
1	7.3, 7.4	0.67
2	14.7, 14.8	0.34
3	18.4, 18.8	1.08
4	34.8, 34.9	0.14
5	35.7, 36.0	0.42
6	35.8, 36.3	0.69
7	36.3, 36.8	0.68
8	40.9, 41.0	0.12
9	75.0, 75.6	0.40
10	95.9, 96.0	0.05
	Mean	0.46

Table II. Correlation of Chemical Analysis with Bioassay

Sample	Tetraethyl Pyrophosphate by Chemical Analysis, %	Bioassay *vs.* Pure TEPP, %
A	89.7	91
B	83.0	88
C	55.0	52
D	52.3	70
E	50.3	44
F	42.3	42
G	41.6	44
H	40.5	42
I	40.5	34
J	37.4	38
K	35.2	32
L	29.2	28
M	15.0	12
N	13.8	12
O	7.4	5
P	5.6	8
Q	0.9	1
R	0.7	1

CHEMICAL ANALYSIS *vs.* BIOASSAY

A large number of samples of hexaethyl tetraphosphate and technical tetraethyl pyrophosphate, including both laboratory preparations and commercial samples, were analyzed by the method outlined and also biologically, using 3rd instar milkweed bugs as the test insect and distilled tetraethyl pyrophosphate (assaying 98.5 to 99.0%) as a comparison standard. Table II illustrates the correlation between bioassay and tetraethyl pyrophosphate content by chemical analysis.

ACKNOWLEDGMENT

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Gravimetric Determination of Thallium with Tetraphenylarsonium Chloride

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A satisfactory gravimetric determination of thallium may be accomplished by precipitation with tetraphenylarsonium chloride. The thallium must be in the trivalent state and in the presence of an excess of hydrochloric acid when the precipitant is added. Hydrogen peroxide serves well to oxidize the thallous ion. The precipitate is washed with hydrochloric acid solution, as distilled water causes some hydrolysis, and is dried to constant weight in an oven at 110° C.

THE methods for determining thallium were reviewed recently by Chrétien and Longi (*1*), who concluded that of fifteen gravimetric methods only the determination as thallous chromate (*5*) is worth retaining. The solubility of thallous chromate in water is so great that the precipitation and washing must be carried out under very strictly standardized conditions if reproducible results are to be obtained.

A method for determining rhenium depends on the formation of an insoluble perrhenate according to the following equation:

$$(C_6H_5)_4As^+ + ReO_4^- \longrightarrow (C_6H_5)_4AsReO_4$$

In an examination of the method (*7*) Willard and Smith found that several ions may interfere, especially if the concentrations are appreciable: permanganate, periodate, perchlorate, thiocyanate, nitrate, iodide, bromide, fluoride, and the complex halide anions which can be formed from some cations. Cations that form insoluble chlorides also interfere.

When trivalent thallium is present in solution with an excess of hydrochloric acid, a white precipitate is obtained on adding the tetraphenylarsonium ion. The weight of precipitate is in excellent agreement with the formation of an insoluble tetraphenylarsonium chlorothallate:

$$(C_6H_5)_4As^+ + TlCl_4^- \longrightarrow (C_6H_5)_4AsTlCl_4$$

The filtrate obtained gives a negative test for thallium with ammonium sulfide (*6*) and with 10% potassium iodide (*2*). Although the chlorothallate ion is apparently present in solid chlorothallic acid (*4*), no other compounds containing this ion have thus far been isolated. The interfering ions are those mentioned above and in addition the perrhenate ion.

REAGENTS

0.1 *N* sodium hydroxide; concentrated hydrochloric acid; solution of 6.7 grams of tetraphenylarsonium chloride in 100 ml. of solution (*7*); 0.1 *N* potassium permanganate; and 30% hydrogen peroxide.

THALLIUM SAMPLES

C.P. thallous sulfate was dissolved, filtered, and recrystallized twice from water.

Thallous perrhenate was prepared by mixing equivalent amounts of thallous sulfate and potassium perrhenate in solution. The relatively insoluble thallous perrhenate was successively recrystallized from water until a reproducible melting point was obtained for the salt.

All samples analyzed were dry weight samples of the above two salts.

PROCEDURE

The thallous compounds were oxidized with 2 ml. of hydrogen peroxide in the presence of sodium hydroxide solution, the mixture was then acidified with hydrochloric acid, and an excess of the acid was added. At this point a white precipitate, apparently a thallous-thallic complex (*4*), forms, but redissolves after the addition of 1 ml. of hydrogen peroxide to the acid solution. Some of the samples were oxidized with standard permanganate solution in the presence of hydrochloric acid (*3*), in order to obtain a check on the thallium content. Care was taken to leave no excess of permanganate in the solution, as the permanganate ion interferes. The solutions were made from 0.5 to 2 *N* with respect to hydrochloric acid and then the solution containing an excess of tetraphenylarsonium chloride was added. The precipitation mixtures at this point varied in volume from 25 to 75 ml. The mixtures obtained were heated to boiling to coagulate the white precipitates and then allowed to stand overnight before filtering on sintered-glass funnels. These precipitates were dried in an oven at 110° C.

The samples that were washed with distilled water all weighed from 0.8 to 2.6 mg. less than the theoretical. The average deviation was approximately 1% low for these samples. Examination of these determinations indicated that the low values were probably not due to impurities in the standard samples, inasmuch as the deviations were independent of sample size. The solubility of the precipitate or the concentration of the acid did not account for the difference, as the volumes and the hydrochloric acid concentrations were each varied by about three times without resulting in a consistent deviation of the weight of precipitate. One of these precipitates, when rewashed with 100 ml. of distilled water, lost several milligrams in weight, and a faint brownish color, very much like a trace of thallic hydroxide, stained the white precipitate. This indicates that the low values were due to hydrolysis of the thallium compound on washing. Subsequently all precipitates were washed with approximately 1 *N* hydrochloric acid; no loss of weight on successive washing was observed. The volume of wash liquid employed varied

Table I. Gravimetric Determination of Thallium

Sample	Sample Wt., Mg.	Oxidizing Agent	HCl Concn., *N*	Volume of Std. $(C_6H_5)_4AsCl$, Ml.	Calcd. Wt. of Ppt., Mg.	Ppt. Found, Mg.	Ppt. Found − Calcd., Mg.	Wash
Tl_2SO_4	25.8	$KMnO_4$	1.0	5	74.5	71.9	−2.6	H_2O
	99.6	H_2O_2	2.0	10	287.8	285.7	−2.1	H_2O
	45.4	$KMnO_4$	1.3	5	131.2	129.4	−1.8	H_2O
	47.9	H_2O_2	1.0	5	138.4	137.2	−1.2	H_2O
	115.4	$KMnO_4$	1.0	10	333.5	332.1	−1.4	H_2O
	59.6	$KMnO_4$	2.0	5	172.2	171.4	−0.8	H_2O
	33.1	H_2O_2	1.5	10	95.7	96.1	+0.4	1 *N* HCl
	99.7	H_2O_2	1.0	10	288.2	289.4	+1.2	1 *N* HCl
	50.9	H_2O_2	0.5	10	147.1	147.1	0.0	1 *N* HCl
	87.5	H_2O_2	0.7	10	252.9	253.1	+0.2	1 *N* HCl
$TlReO_4$	409.7	$KMnO_4$	0.7	25	1228.3	1220.8	−7.5	H_2O
	58.2	H_2O_2	2.0	10	174.5	174.4	−0.1	1 *N* HCl
	194.7	H_2O_2	0.5	10	583.7	583.8	+0.1	1 *N* HCl

from 20 to 40 ml. The average deviation from the theoretical weight was 0.1% for precipitates that were washed with hydrochloric acid solution.

The factor 2.890 converts weight of thallous sulfate to tetraphenylarsonium chlorothallate. The weight of thallous perrhenate is converted to the equimolecular mixture of tetraphenylarsonium perrhenate and tetraphenylarsonium chlorothallate by the factor 2.998. Thallium was not separated from rhenium but was simultaneously precipitated with tetraphenylarsonium chloride.

SUMMARY

Thallium may be quantitatively determined by precipitation from hydrochloric acid solutions containing the element in the trivalent state. The oxidation of the thallous ion may be accomplished with hydrogen peroxide or any other effective oxidizing agent which introduces no interfering substance. The precipitating reagent is a water solution of tetraphenylarsonium chloride. The amount of excess of this reagent is not critical. The precipitate obtained should be washed with hydrochloric acid solution to prevent hydrolysis, and dried in an oven at 110° C.

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Determination of Alkyl Sulfides and Disulfides

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A procedure for determining alkyl disulfides, which was found to work equally well for alkyl sulfides, involves oxidation with bromine. Alkyl sulfides and disulfides can be determined in the presence of each other by the application of a second method. Samples containing less than 10 mole % thiol can also be determined. The procedure is precise to about ±0.3% in the best cases and goes as low as ±1.0% in samples where the end point is rather slow.

KOLTHOFF *et al.* (*4*) devised a method for determining alkyl disulfides which involves reduction to the corresponding thiols (mercaptans) and amperometric titration of the thiols with silver nitrate. This method has the disadvantage of being reproducible to only about 2% in the optimum range of sample size and of yielding results that are about 5% low.

A procedure yielding a higher precision and accuracy was sought, and the following oxidation method was devised. The method described involves oxidation of the disulfide with bromine to the formation of the corresponding sulfonyl bromide.

$$RSSR + 5Br_2 + 4H_2O \longrightarrow 2RSO_2Br + 8HBr \qquad (1)$$

The oxidation is brought about by adding standard bromate-bromide solution to an acid solution of the disulfide. As soon as the bromate-bromide strikes the acid solution, the bromine is liberated and is consumed by the disulfide. The end point is taken as the appearance of the first permanent bromine coloration. The usual method of adding excess bromate-bromide and determining the excess reagent iodometrically cannot be used in determining disulfides because of substitution reactions which consume bromine and cause high results. The substitution reactions do not interfere as long as there is no excess bromine present.

This same procedure was found to be applicable to the determination of alkyl sulfides. The reaction is the oxidation of the sulfide to the sulfoxide.

$$R_2S + Br_2 \longrightarrow R_2S.Br_2 \xrightarrow[H_2O]{} R_2SO + 2\,HBr \qquad (2)$$

In this case, as with the disulfides, using an excess bromate-bromide and determining the excess cause high results. In the case of the sulfides, the trouble is not only substitution but further oxidation of the sulfoxide to the sulfone. Neither of these reactions interferes as long as excess bromate-bromide is avoided. A similar procedure was used by Sampey, Slagle, and Reid (*5*), who titrated the sample, in benzene solution, with a solution of bromine in water. The main disadvantage of this method lies in the fact that bromine water is not a very stable standard solution. Bromine vapors are continually lost from the solution.

Mixtures of alkyl disulfides and sulfides can be determined by first applying the bromine oxidation, which yields both the disulfide and the sulfide. Then, by using the method of Kolthoff *et al.* (*4*) the disulfide alone can be determined; the sulfide is then obtained by difference. The extent of the application of this method is limited by the relatively low precision and accuracy of the reduction method, and by the fact that the final result depends on a subtraction and that the disulfide oxidation involves 5 moles of bromine while the sulfide oxidation involves 1 mole. The best results are obtained on samples low in disulfide and high in sulfide; here, the error in the disulfide result does not figure too prominently in the final results.

Thiols are also oxidized by bromine and act as interferences if they are not determined separately and the results corrected for their presence.

$$2RSH \xrightarrow{Br_2} RSSR \xrightarrow[H_2O]{5Br_2} 2RSO_2Br$$

This procedure can be used to determine thiols. The precision is high (±0.5%), but the accuracy is low. When pure ethanethiol was run by this method, the results were very precise but were 3% low. 1-Pentanethiol results were 8% low. However, if the sulfide or disulfide contains thiol equivalent to 10% or less of the total titration, the absolute error in the first result is not significant. For instance, if the sample contains ethanethiol equivalent to 10% of the total titration, then the error in the total analysis is only 0.3%. In the case of 1-pentanethiol, the error would be 0.8%. The error introduced by the thiol increases as the amount of thiol in the sample increases.

Samples of disulfide or sulfide containing thiols are determined by the bromination procedure described below, which yields both the thiol and the disulfide or sulfide; the thiol alone can be

Table I. Sulfides

	Concd. Acid Used, Ml.	%
n-Butyl sulfide in 50 ml. of 80% HAc–20% H_2O	3 HCl	99.5
	13 HCl	99.0
	25 H_2SO_4	99.5
Benzyl sulfide[a] in 50 ml. of 80% HAc–20% H_2O	3 HCl	99.5
		100.3
		100.1
Ethyl sulfide in 100 ml. of 80% HAc–20% H_2O	3 HCl	98.3
		99.0
		99.1
		98.3

[a] Sulfur analysis was run on this sample: % S found = 15.20, % S calcd. = 14.95.

Table II. Disulfides

	Concd. Acid Used, Ml.	%
Ethyl disulfide in 50 ml. of 80% HAc–20% H_2O	3 HCl	71.3
	10 HCl	86.4
	20 H_2SO_4	95.6
	25 H_2SO_4	95.3
	30 H_2SO_4	95.3
Butyl disulfide in 50 ml. of 80% HAc–20% H_2O	3 HCl	87.2
	5 HCl	88.7
	10 HCl	95.8
	25 HCl	96.8
	5 6 *N* H_2SO_4	79.6
	5 12 *N* H_2SO_4	83.0
	10 12 *N* H_2SO_4	86.9
	10 H_2SO_4	97.8
	20 H_2SO_4	98.8
	25 H_2SO_4	98.8
	30 H_2SO_4	98.8
Butyl disulfide in 25 ml. of 80% HAc–20% H_2O	10 H_2SO_4	98.8
Phenyl disulfide[a] in 50 ml. of 80% HAc–20% H_2O	3 HCl	94.5
		95.0
		94.3
	25 HCl	94.0
	20 H_2SO_4	Consumption of Br_2 too slow for good end point
1-Cystine[a] in 50 ml. of water	3 HCl	100.0
		99.6
	25 HCl	99.6
	20 H_2SO_4	99.6

[a] Sulfur analyses were run on these samples.
Phenyl disulfide, % S found = 27.94; % S calcd. = 29.4.
1-Cystine, % S found = 26.87; % S calcd. = 26.6.

determined by the amperometric titration method of Kolthoff and Harris (*3*).

REAGENTS

0.1 *N* Bromate-Bromide. After 2.78 grams of dry potassium bromate and 10 grams of potassium bromide are dissolved in water and diluted to 1 liter, the solution is standardized by putting 50 ml. of bromate-bromide solution in a 250-ml. iodine flask. To the solution are added 6 ml. of 6 *N* sulfuric acid; no bromine vapors are allowed to escape. The flask is chilled, and 10 ml. of the 20% potassium iodide are added via the well around the glass stopper of the flask. Precautions must be taken to prevent escape of bromine. The flask is shaken to account for the bromine in the atmosphere of the flask. The liberated iodine is then titrated with 0.1 *N* thiosulfate, using starch indicator.

Glacial acetic acid, C.P. concentrated hydrochloric or sulfuric acid.

PROCEDURE

A sample containing about 0.001 to 0.002 mole of alkyl sulfide or about 0.0003 mole of alkyl disulfide is weighed into a 250-ml. Erlenmeyer flask and dissolved in 40 ml. of glacial acetic acid. To this solution are added about 10 ml. of water (less water can be added if the sample comes out of solution). In the case of alkyl sulfides, 3 ml. of concentrated hydrochloric acid are sufficient to achieve complete reaction (see Table I). In the case of the alkyl disulfides, 3 ml. of concentrated hydrochloric acid are usually insufficient and more acid is generally required; 25 ml. of either concentrated hydrochloric acid or concentrated sulfuric acid will cause the reaction to proceed completely (see Table II). After the acid has been added, the contents of the flask are titrated with standard 0.1 *N* bromate-bromide solution until the bromine color persists. The end point can be noted within 2 drops of reagent. A blank should be run on the acetic acid–water mixture used as solvent to correct for the excess bromine needed for the end point. This blank is subtracted from the titration; the blank is small, but it is still significant.

For optimum results the disulfide samples should be slightly warm during the titration (30° to 50° C.). If the solution is at room temperature, the end point is a little slow, and a slight error can be incurred. The sulfide samples can be titrated at room temperature.

DISCUSSION

The analyses require very little time and a minimum of apparatus. The end point is not of the sharpest type, but is easily discernible; by using samples that yield a 30-ml. titration, the titration can be reproduced to 0.5% or better. The blank which accounts for the amount of excess bromine needed to see the end point is about 0.25 ml.

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Determination of Moisture in Lecithin and Crude Soybean Oils

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THE determination of moisture in commercial lecithin presents many problems. Thermal drying and toluene distillation methods are not reliable, although the latter method is in general use at present (*4*). The specific disadvantages of the toluene distillation method when applied to lecithin are excessive foaming in the boiling flask and poor phase separation in the water trap. Results thus obtained may vary as much as 20% (*6*).

As lecithin is a material of relatively low water content, it was thought desirable to investigate the Karl Fischer method. This paper presents an accurate and rapid method for the determination of water in lecithin. The procedure described employs the Karl Fischer reagent with the determination of the end point by the dead stop method (*18*).

The Fischer reagent has been widely used for a variety of products (*1–3, 5, 7–13, 15–19*). The limitations of the method have been discussed by Mitchell *et al.* (*14*) and by Suter (*16*). By careful attention to the end-point approach, and by elimina-

A rapid and accurate method is described for the determination of moisture in lecithin and crude soybean oils with the Karl Fischer reagent. The precision is found to be ±0.4 and ±0.9% of the water determined in lecithin and crude soybean oil, respectively. Application of this method to corn oil and to hexane–soybean oil mixtures is also mentioned.

tion of a solvent correction, the method may be applied to lecithin with very satisfactory results.

APPARATUS

The Precision Aquatrator made by the Precision Scientific Company was used in this investigation. This apparatus consists of two autoburets, double platinum electrodes, Bakelite beaker cover with moistureproof seal for buret tip, stirrer, and electrodes, and an extremely sensitive galvanometer for determination of the end point by the dead stop method. The glass sample container has a capacity of 100 ml. and is equipped with a No. 45/12 male taper. No batteries are employed in the electronic circuit, and the meter operation is not affected by ordinary fluctuations in line voltages.

REAGENTS

Fischer Reagent. Both commercial (Eimer & Amend) and laboratory preparations were used in this investigation. The laboratory reagent was prepared according to the directions of Johnson (*11*). Owing to the initial rapid deterioration, the reagent was allowed to age at least 2 days before using.

Standard Water-Methanol Solution. Add 4 ml. of water to 1 liter of commercial absolute methanol to give a solution containing about 5 mg. of water per ml.

Solvent. Add 3 parts of dry chloroform to 1 part of dry methanol. Technical chloroform was dried over 6- to 16-mesh silica gel for 3 to 4 days (*1*). Commercial absolute methanol was further dehydrated by refluxing over magnesium ribbon. Both solvent components had a water content of less than 0.01%.

Table I. Precision of Determination of Water in Lecithin

Water Found, %	Deviation from Mean, %
0.870	+0.005
0.866	+0.001
0.865	0.000
0.862	−0.003
0.868	+0.003
0.864	−0.001
0.860	−0.005
0.868	+0.003
Mean 0.865%	

PROCEDURE

Place 50 ml. of solvent in a clean, dry sample container, add 1 to 2 ml. of Fischer reagent, and back-titrate with the standard water-methanol solution. Allow the titrated solvent to remain in operating position on the container support until ready for use.

Weigh accurately about 5 grams of lecithin into a second clean, dry sample container. Run in about 15 ml. of the Fischer reagent and immediately add the titrated solvent. Place in operating position and stir for 2 to 3 minutes to disperse the lecithin completely. Slowly add water-methanol until the first slight shift of the galvanometer needle is noted. Close the buret, allow to stir for 30 seconds, and proceed with the titration, dropwise, until the galvanometer needle comes to its rest point. This end titration must be done with care, allowing at least a 30-second stirring time between drops. The characteristic lag near the end point is especially evident in this titration. However, the end titration is not particularly time-consuming, as only 3 to 5 drops of the water-methanol are needed after the first slight shift of the galvanometer needle.

Standard methods were used to calculate the water content and to standardize the reagents (*18*, *19*).

In applying the procedure to crude soybean oils, a 20- to 25-gram sample is used. All other steps are the same.

PRECISION

Several determinations were made on a stock sample of lecithin (Table I). The maximum deviation from the mean is ±0.005%, and the average deviation is ±0.003%, which is equivalent to ±0.4% of the water determined.

ACCURACY

Recovery of known amounts of water added to lecithin was used to check the accuracy of the method. The same stock sample of lecithin was used as in the precision evaluation; the average of the values given in Table I was taken as the initial water content. Water was added from a weight buret to weighed samples of lecithin contained in the 100-ml. sample containers. The samples were allowed to stand a few minutes to permit the lecithin to absorb the added water, and the analyses were made as usual. Table II gives the results of these experiments. The maximum deviation from the average water found was ±0.9%. The average deviation, however, is only ±0.35%, which makes the accuracy of the determination at this level equal to the precision as shown in Table I.

APPLICATION TO OTHER MATERIALS

The method has also been applied to both crude and refined corn and soybean oil. Data for crude soybean oil are given in Table III. The maximum deviation from the mean is ±0.002%, and the average deviation is 0.001%, which is equivalent to ±0.9% of the water determined. This precision is considered satisfactory in view of the small quantity of water involved.

Hexane–soybean oil mixtures were also examined. The presence of hexane necessitated a slight change in the procedure because of its immiscibility with Fischer reagent. The presence of 10 to 20 ml. of hexane in the sample for analysis required the addition of a large excess of Fischer reagent to obtain satisfactory operation of the galvanometer. However, the back-titration proceeded smoothly, and the results were satisfactorily reproducible. In combination with conventional oven-drying methods for vegetable oils, it is possible to obtain, and distinguish between, water and volatile matter nonwater constituents.

Table II. Recovery of Added Water from Lecithin

Weight of Sample, Grams	Water Originally Present, Gram	Water Added, Gram	Total Water Present, Grams	Total Water Found, Gram	Water Calculated, %	Water Found, %	Recovery, %
6.023	0.0521	0.0628	0.1149	0.1149	1.908	1.908	100.0
7.468	0.0646	0.0722	0.1368	0.1367	1.830	1.828	99.9
6.786	0.0587	0.0700	0.1287	0.1293	1.897	1.905	100.4
3.926	0.0340	0.0694	0.1034	0.1047	2.634	2.666	101.2
5.677	0.0491	0.0350	0.0841	0.0845	1.481	1.488	100.5
4.495	0.0389	0.0692	0.1081	0.1078	2.405	2.398	99.7
4.639	0.0401	0.1066	1.1467	0.1467	3.162	3.162	100.0
4.915	0.0425	0.0672	0.1097	0.1100	2.232	2.338	100.0
						Av.	100.3

Table III. Precision of Determination of Water in Crude Soybean Oil

Water Found, %	Deviation from Mean, %
0.112	0.000
0.113	±0.001
0.110	−0.002
0.111	−0.001
0.113	+0.001
Mean 0.112%	

SUMMARY

The Karl Fischer reagent has been employed in the development of an accurate and rapid method for the determination of moisture in lecithin. Difficulties presented by previous methods for this determination have been entirely eliminated. The precision has been found to be ±0.4% of the water determined. The method is also applicable to related materials such as crude and refined corn and soybean oils.

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Spectrographic Determination of Boron in Plant Tissue

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A spectrographic procedure of good precision for the determination of boron in plant ash employs the direct current arc for excitation. Tin is used as an internal standard and lithium chloride as a buffer. The plant material is ashed at 600° C. A weighed portion of ash is treated with hydrochloric acid, evaporated to dryness, and taken up in a solution of internal standard and buffer. A 0.1-ml. aliquot is evaporated to dryness on the electrode. The sample is burned in a direct current arc with a blunt-nosed cathode. Line densities are determined with a densitometer and compared with those produced by standards of known concentration. Blunt-nosed cathodes are preferred for increased precision. A probable error of 1.43% was obtained with alfalfa samples containing 36.6 p.p.m. of boron. With pointed electrodes a probable error of 2.70% was obtained with samples containing 28.4 p.p.m. The procedure agrees well with data obtained by the use of quinalizarin as a color-producing reagent. In nine alfalfa samples of varying boron content, spectrographic analyses averaged 6.3% lower than the colorimetric procedure. The method has also been successfully used on wheat grain. The simultaneous estimation of phosphorus, manganese, and magnesium by this procedure is possible.

EXTENSIVE literature on the subject of boron as an essential element in proper plant growth and development indicates that a knowledge of the boron content of plant materials is of major importance. Inasmuch as boron, although essential, is present in relatively small quantities, this element requires a procedure that is sensitive at low concentrations.

Two general methods of analysis for such a case are suggested, colorimetric and spectrographic. In by far the largest portion of the work reported in the literature on the boron content of plant materials, quinalizarin has been used as a color-producing reagent. Berger and Truog (*1*) in particular have studied the development of this procedure. Curcumin and turmeric also have been suggested as color-developing reagents for the determination of boron. Some work on the development of a spectrographic method of analysis for boron in plant materials has been done by McHargue *et al.* (*4*). Parks (*8*) has reported on the determination of boron in synthetic soil mixtures, and Melvin and O'Connor (*5*) have investigated the spectrochemical analysis of fertilizers for boron and other trace elements.

Although capable of giving fair results, most of the above-mentioned procedures have certain disadvantages. The quinalizarin reaction must be carried out in 98% sulfuric acid and the acid concentration is critical. The shift in the absorption maximum of the reagent, which is produced by the boron, is small, about 40 mμ (*11*). This may lead to difficulty with certain filters in the usual type of colorimeter employed for this purpose. The use of curcumin or turmeric (*7*) gives results that agree satisfactorily with other procedures (*1*, *3*). Results on the titration procedure of Wilcox (*12*) have been shown to be relatively high on small quantities of boron (*1*).

The procedure of McHargue *et al.* (*4*) consisted in converting the boron in plant material to methyl borate, distilling off the methyl borate, and determining the boron in the distillate spectrographically. These authors also reported on the determination of boron in the plant ash directly but without the added controls of a spectrographic buffer and an internal standard. The advantages of spectroscopic procedures suggested that a further study of this method might lead to modifications for stabilizing the boron line and inhibiting the effects of the extraneous elements. The following spectrographic procedure gives high accuracy and reproducibility with a minimum of preliminary work. Results compare favorably with the quinalizarin procedure. Furthermore, although boron was the object of the study, the conditions under which the spectra are produced will permit the simultaneous estimation of phosphorus, manganese, and magnesium with no further change in conditions.

PROCEDURE

The following method has been used successfully with the Bausch & Lomb large Littrow spectrograph.

Reagents and Materials. Eastman type 33 spectrographic plates and Eastman x-ray developer and fixer are used.

Previously prepared standards approximating the composition of plant ash contain varying amounts of boron.

An internal standard is prepared by dissolving reagent quality tin in sufficient hydrochloric acid to keep it in solution. This is

diluted so that the final concentration of tin becomes 1 mg. per ml.

A spectrographic buffer consists of lithium chloride dissolved in 1 to 2 hydrochloric acid, so that a final concentration of 5 grams of lithium chloride per 100 ml. of solution is obtained.

Preparation of Standards. A series of standards was made up from a stock solution that contained 0.010 gram of boron per liter after diluting with buffer. Included in the standards were potassium, phosphorus, calcium, magnesium, iron, sodium, and copper in amounts that approximated the composition of the plant ash. These standards were added in varying amounts to a buffer solution. One milliliter of internal standard was added in each case. The final solutions all had a volume of 4 ml. With the exception of the internal standard and the use of the lithium chloride buffer, the solutions were similar to those suggested by Morris *et al.* (*6*).

Spectrographic Procedure. Two grams of the dry plant tissue were ashed in a porcelain crucible at 600° C. The percentage of ash was noted and 30 mg. of the ash were weighed into a small glass vial. One or 2 ml. of 1 to 1 hydrochloric acid were added and the solution was evaporated just to dryness. The residue was cooled and then taken up in 3 ml. of buffer and 1 ml. of internal standard solution and mixed thoroughly.

Electrodes were cut from carbon electrodes $^5/_{16}$ inch in diameter sold by the National Carbon Company under the name of special carbon spectroscopic electrodes. The anode was shaped with a shallow cone crater about 1.5 mm. in depth. The cathodes were shaped like a round-nosed bullet. Electrodes were made impervious to the sample solution by pre-treatment with a saturated solution of carnauba wax in carbon tetrachloride. The craters were filled to excess with the solution, dried in an air oven for 15 minutes at 100° C., and allowed to cool. With the aid of a serological pipet 0.10-ml. aliquots of the unknown or standard solution were placed in the craters of each of two electrodes. The electrodes were next placed in an air oven at 80° C. for 15 minutes; then the temperature was raised to 100° C. for several hours, preferably overnight, after which the electrodes were ready for arcing.

The samples were arced at 120 volts and 8 amperes for 45 seconds with the sector set at five-eighths open, equivalent to 14 seconds' exposure. Then, without moving the plate, an identical exposure was made at the same position on the plate with the duplicate electrode. Arc spacing was maintained at 5 mm. and wandering of the arc was controlled manually.

After exposure the plates were developed for 5 minutes at 20° C. (68° F.) in Eastman x-ray developer, diluted 2 to 1, fixed, and dried, and line densities were determined with the aid of an ARL-Dietert densitometer. Uniformity of plates and exposure could be checked by comparing the six standards taken on each plate with previously prepared calibration curves. Little variation occurred between plates.

RESULTS AND DISCUSSION

The method described has been applied successfully to the determination of boron in alfalfa (*10*) and in wheat (*9*). Alfalfa, with its relatively high boron content, gave good results. In order to determine boron in wheat at its lower level of about 1 p.p.m. the size of the ash sample was increased to four times that used for alfalfa. Results, with this as the only modification, were satisfactory. Samples burned uniformly and the arc seemed more stable with the lithium chloride buffer than with any of the many other buffer materials that were tried.

Selection of a Suitable Buffer. Parks (*8*) has reported on a number of buffers for use in the determination of boron in synthetic soils. He has shown the necessity of careful selection of a good buffer in order to limit the effects of extraneous elements and give suitable calibration curves. Brunstetter and Myers (*2*), however, report satisfactory results on boron and other elements in plant tissue without buffers or internal standard. As a proper selection of buffer and internal standard is generally believed to give more satisfactory results, this procedure was adopted.

Data of Parks (*8*) were confirmed regarding the effects of aluminum chloride, sodium chloride, and ammonium chloride. Calibrations of standard solutions using these materials as buffers are shown in Figure 1. The effect of sodium sulfate is also shown. It is obvious that straight-line calibrations do not occur with aluminum chloride and sodium chloride. The other two salts are better. Calcium chloride, as a buffer, markedly suppressed the boron line and was rejected. Further work in this regard led to the use of lithium chloride as a buffer. This material, in addition to having properties which produce a straight-line calibration with boron, helps maintain a steady arc during excitation. Its tendency to absorb moisture is circumvented by leaving the electrodes in the oven until just prior to arcing.

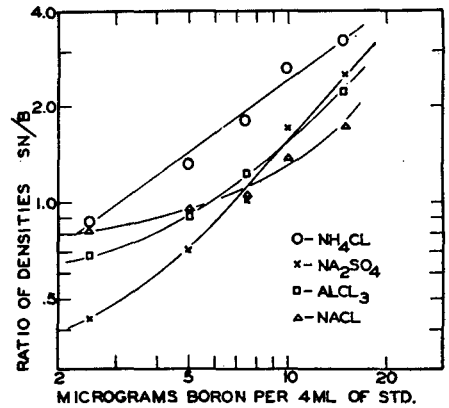

Figure 1. Effects of Buffer Materials on Boron-Tin Calibration Curve

Selection of Internal Standard. A number of lines have been suggested for use as an internal standard for the determination of boron. Parks (*8*) has reported that the tin line at 2495.7 Å. is satisfactory and possesses characteristics desirable in an internal standard when the excitation source is a high voltage alternating current arc. His work was verified for the direct current arc used in this work; and consequently, tin was selected for the data reported here. As the boron line used has a wave length of 2497.8, the boron and tin lines are close togeher, and danger of error due to emulsion differences are minimized. Background effects were a minimum and no correction for background was required. The working curve is a straight line with approximately a 45° slope when plotted in the usual manner for spectrographic calibrations.

Table I. Analyses of Alfalfa Plant Ash for Precision of Spectrographic Determination of Boron

Type of Cathode	No. of Analyses	Av.	Extreme Values	Standard Error	Probable Error	Probable Error
		P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	%
Blunt-nosed	11	16.3	14.7–19.0	0.402	0.28	1.73
	12	36.6	32.5–40.8	0.747	0.52	1.43
Sharp-pointed	10	28.4	24.0–33.6	1.09	0.77	2.70

PRECISION OF METHOD

Table I presents data that indicate the precision of the method described under different concentrations of boron. For part of this study two samples of alfalfa ash of different boron concentrations were used with the round-nosed electrodes. For comparison purposes alfalfa samples of a higher level of boron concentration were also analyzed several times, using sharp-pointed electrodes; averages were obtained and the deviations and probable errors were calculated. In case of the sample containing the smaller amount of boron, the probable error was 1.73%. In the second case, where the higher level sample was used in conjunction with the round-nosed electrodes, a probable error of 1.43% was calculated. On a sample using the sharp-pointed electrodes a probable error of 2.70% was calculated. This indicates the greater stability obtained by the use of the round-nosed electrodes. Further information regarding precision may be obtained from

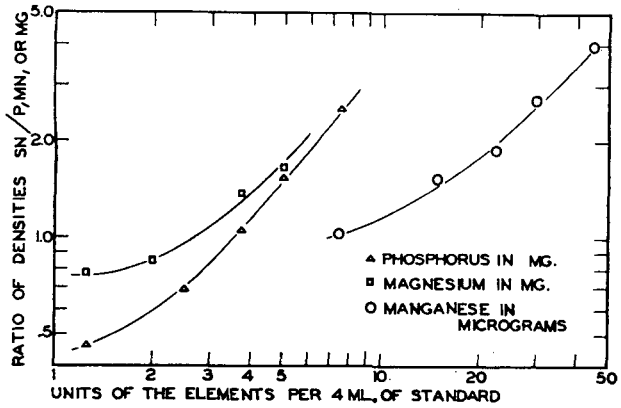

Figure 2. Calibration Curves for Phosphorus, Magnesium, and Manganese

Table II. Comparison of Spectrographic and Quinalizarin Methods for Analysis of Boron

Sample	Quinalizarin *μg./sample*	Spectrograph *μg./sample*	Difference %
1	5.5	4.7	−14.5
2	7.4	7.6	2.7
3	8.6	7.8	−9.3
4	8.7	7.7	−6.7
5	9.0	8.4	−11.5
6	11.5	11.7	1.7
7	12.2	13.3	9.0
8	13.0	11.4	−12.3
9	16.8	14.0	−16.2

the calculation of the probable errors of the spectrographic determinations reported in Table II. The mean probable error calculated for these samples, analyzed in duplicate, is 4.1%.

COMPARISON WITH QUINALIZARIN PROCEDURE

The spectrographic procedure described was compared with the quinalizarin procedure which is now commonly used. The data presented in Table II are the average of duplicate determinations in both the spectrographic and colorimetric procedures. Agreement between the methods is good. The spectrographic results average approximately 6.3% less than those obtained by the colorimetric procedure. The maximum difference between the two methods was 16.2%.

DETERMINATION OF OTHER ELEMENTS SIMULTANEOUSLY WITH BORON

Although the procedure has been developed for boron and analyzed with respect to the reliability of boron determinations, Figure 2 indicates the possibility of the simultaneous determination of phosphorus, manganese, and magnesium. The same tin line used for boron served as the internal standard for these elements. Spectral lines used were phosphorus, 2534.0 Å.; manganese, 2801.1 Å.; and magnesium, 2790.8 Å. These calibration curves are in the range of concentrations usually found in plant materials, and while it is to be expected that probable errors would be larger than in the case of boron, it is also likely that the data so obtained would be very useful.

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Elimination Maxima of Certain Saturated Cholesterol Esters

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Pure cholesterol and a series of saturated cholesterol esters were prepared and subjected to analytical molecular distillation. The data of the distillations were plotted and the elimination maxima were estimated. The temperature point at which 50% of the sterol has distilled, called the ET50, is suggested as a new reference point for the molecular distillation of a compound. The addition of two CH_2 groups in the ester chain increased the elimination maximum (or ET50) of the cholesterol esters approximately 10° C.

ALTHOUGH several investigators (*2, 5, 8, 11*) have indicated that cholesterol esters should seriously be reckoned with in any consideration of fat metabolism, the study of the functions of these esters has been hampered by difficulties in the separation and analysis of the component fatty acids. In the hope that analytical molecular distillation may circumvent some of the existing difficulties, the elimination maxima of certain saturated esters have been obtained. The rationale of the present approach is simply that if these maxima are known for a series of esters, an unknown ester might be identified by comparison of the elimination curves.

Molecular distillation is a technique of high-vacuum short-path distillation of heavy oils or low melting solids which otherwise could not be distilled without serious pyrolytic changes. Analytical distillation (*5, 8*) is a quantitative adaptation in which the charge is fractionated by progressive and regularly increasing increments of temperature. The quantity of active ingredient in each fraction, when plotted against the temperature at which it was collected, gives a curve of characteristic shape having a peak at a temperature related to the compound being distilled. The curve is termed the elimination curve, and its peak, the elimination maximum. Because of the size of the apparatus and the small quantity of active ingredient involved, an inert constant yield oil must be used, which provides a constant amount of distillate at

Table I. Sterol Distribution in Fractions

	130° C.	140° C.	150° C.	160° C.	170° C.	180° C.	190° C.	200° C.	210° C.	220° C.	230° C.	240° C.	250° C.	260° C.	Recovery %	Recovery *Mg.*
							Milligrams of Cholesterol									
Cholesterol I	12.3	17.0	40.0	50.2	58.3	45.3	29.3	13.0	4.3	1.7	0.9	..	..	..	90	272
Cholesterol II	13.9	20.0	37.8	50.8	60.2	50.0	28.5	15.6	6.4	2.3	2.2	..	..	..	95	288
Cholesterol III	13.9	21.4	37.8	54.2	65.5	48.5	23.8	11.6	5.6	2.8	2.6	..	..	..	95	288
							Milligrams of Cholesterol Esters									
Acetate I	8.9	18.5	31.2	52.5	63.2	56.2	37.5	17.3	5.2	3.2	2.3	..	..	..	97	293
Acetate II	9.5	14.5	30.0	47.3	57.2	56.4	38.4	17.3	7.2	3.1	2.2	..	..	..	97	283
Butyrate I	4.3	9.2	18.4	41.5	57.5	59.0	53.7	35.2	16.9	7.8	3.8	..	..	..	102	307
Caproate I	1.5	3.6	9.8	19.2	33.5	47.6	55.5	48.8	37.0	21.2	9.4	..	..	..	95	287
Caproate II	2.9	3.2	7.6	16.4	32.2	49.6	61.7	67.6	45.2	24.7	11.5	..	..	..	107	323
Caproate III	0.9	3.3	7.6	15.3	30.0	48.1	60.8	61.6	44.5	24.7	9.6	..	..	..	102	306
Caprylate I	0.8	1.6	3.3	7.5	13.1	21.7	36.0	51.8	50.5	38.8	27.5	..	..	..	84	253
Caprylate II	0.7	1.8	4.5	8.6	17.5	31.9	45.5	58.6	57.5	49.8	32.3	..	..	..	102	309
Caprate I	..	0.9	2.7	6.5	10.3	19.4	34.4	52.9	59.2	57.5	42.6	..	..	..	95	286
Caprate II	..	..	..	..	11.7	16.6	29.3	45.6	57.9	57.5	45.0	24.2	10.9	6.8	101	306
Laurate I	0.7	0.2	1.2	2.9	5.8	10.5	16.4	28.9	41.8	54.7	57.0	46.0	30.8	16.1	104	313
Laurate II	..	..	..	..	6.5	10.8	16.9	28.8	41.5	51.6	55.5	47.5	29.2	16.4	101	305
Myristate I	0.1	0.7	1.6	3.6	5.4	8.5	11.5	17.5	28.3	42.3	51.4	52.6	44.6	35.0	101	303
Palmitate I	..	..	..	2.7	4.5	6.8	8.7	13.6	18.4	31.9	44.5	51.0	52.0	48.7	97	283
														Av.	97.7	

each temperature interval and acts as a carrier for the sample. A residue oil which does not distill over in the temperature range involved supplies the necessary bulk to the charge. A pilot dye (*1*) can also be included, which distills with the sterol and acts as a control for the distillation by providing its own characteristic elimination maximum in each distillation.

EXPERIMENTAL TECHNIQUE

Preparation of Cholesterol and Cholesterol Esters. Pure cholesterol was prepared by crushing and extracting gallstones with 95% ethyl alcohol. The extract was recrystallized twice from ethyl alcohol and the crude cholesterol was further purified by forming dibromocholesterol according to the method of Bills, Honeywell, and MacNair (*2*). The cholesterol was liberated from the dibromo compound by treatment with zinc dust. On recrystallization from ethyl alcohol, white crystalline cholesterol was obtained (melting point 147° C.).

The acetate, butyrate, caproate, caprylate, caprate, laurate, myristate, and palmitate were prepared from cholesterol and the respective fatty acids or the acid anhydrides according to the methods of Page and Rudy (*11*). The melting points of the esters were: acetate, 114.5° C.; butyrate, 104° C.; caproate, 99.0° C.; caprylate, 108.0° C.; caprate, 84.0° C.; laurate, 79.0° C.; myristate, 71.5° C.; and palmitate, 80.0° C.

Preparation of Constant Yield Oil. Benzoylated sterol-free castor oil was prepared as the residue oil. Reagent grade castor oil, which contained appreciable quantities of sterols, was purified by chromatography, using a petroleum ether solution on Doucil (a commercial water softener). The free sterols were adsorbed at the top of the column as a light yellow band with the glycerol triricinoleate in the second band. The sterol esters were washed through by petroleum ether. The top band was removed and the column eluted with chloroform, giving essentially sterol-free castor oil (0.003% by the Liebermann-Burchard reaction). The sterol-free castor oil was benzoylated by treating with benzoyl chloride at elevated temperatures, and the product was purified by stripping in the molecular still.

Pilot Dye. Diamylaminoanthraquinone obtained from Distillation Products, Inc., was used as a pilot dye for each distillation.

Distillation of Esters. A charge consisting of 50 grams of constant yield oil, 100 grams of residue oil, 15 mg. of pilot dye, and 300 mg. of cholesterol or one of the several cholesterol esters was introduced into the still. The analytical distillation was carried out at 1 to 5 microns pressure in a 500-ml. cyclic still built by Distillation Products, Inc. Fractions were collected at 10° C. intervals from 130° to 230° C. and in some cases from 130° to 260° C.

Analysis of Fractions. The sterol content of the fractions was determined by the Liebermann-Burchard reaction (*9*), using a photoelectric colorimeter. Good recoveries and reproducible results were obtained by adding the acetic anhydride and sulfuric acid separately from microburets. [Sperry and Brand (*12*) recommend mixing the acetic anhydride and sulfuric acid to ensure accurate volumes, but for this study it was found satisfactory to add the reagents separately.] Data on recovery of cholesterol and the several esters are given in detail in Table I. The dye content of each fraction was obtained by dilution of each fraction followed by a colorimetric determination.

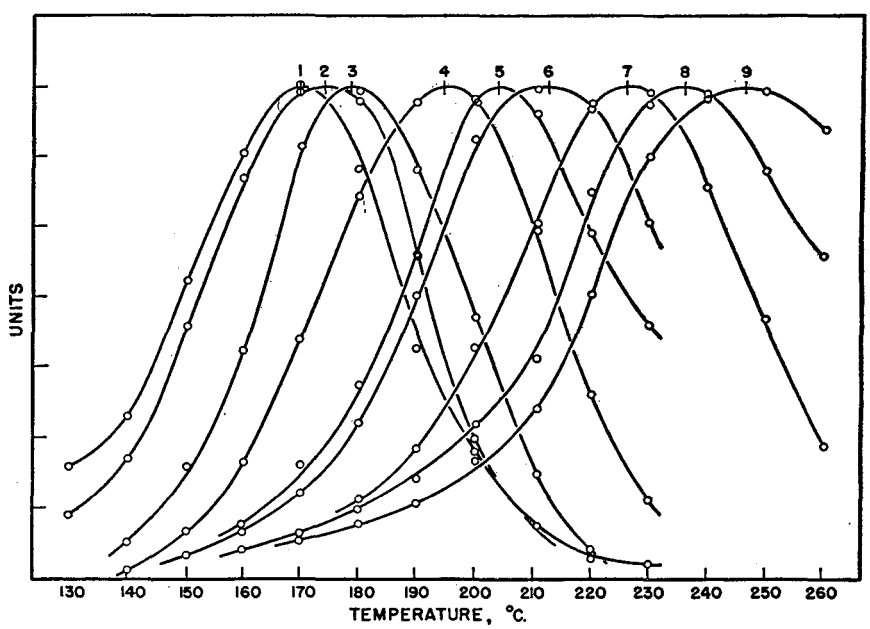

Figure 1. Elimination Maxima

Elimination curves have all been adjusted to same height, for better comparison, by multiplying actual value by an arbitrary constant. The ordinate units are therefore different for each distillation.

1. **Cholesterol, 169° C.**
2. **Acetate, 171° C.**
3. **Butyrate, 178° C.**
4. **Caproate, 196° C.**
5. **Caprylate, 204° C.**
6. **Caprate, 212° C.**
7. **Laurate, 226° C.**
8. **Myristate, 236° C.**
9. **Palmitate, 248° C.**

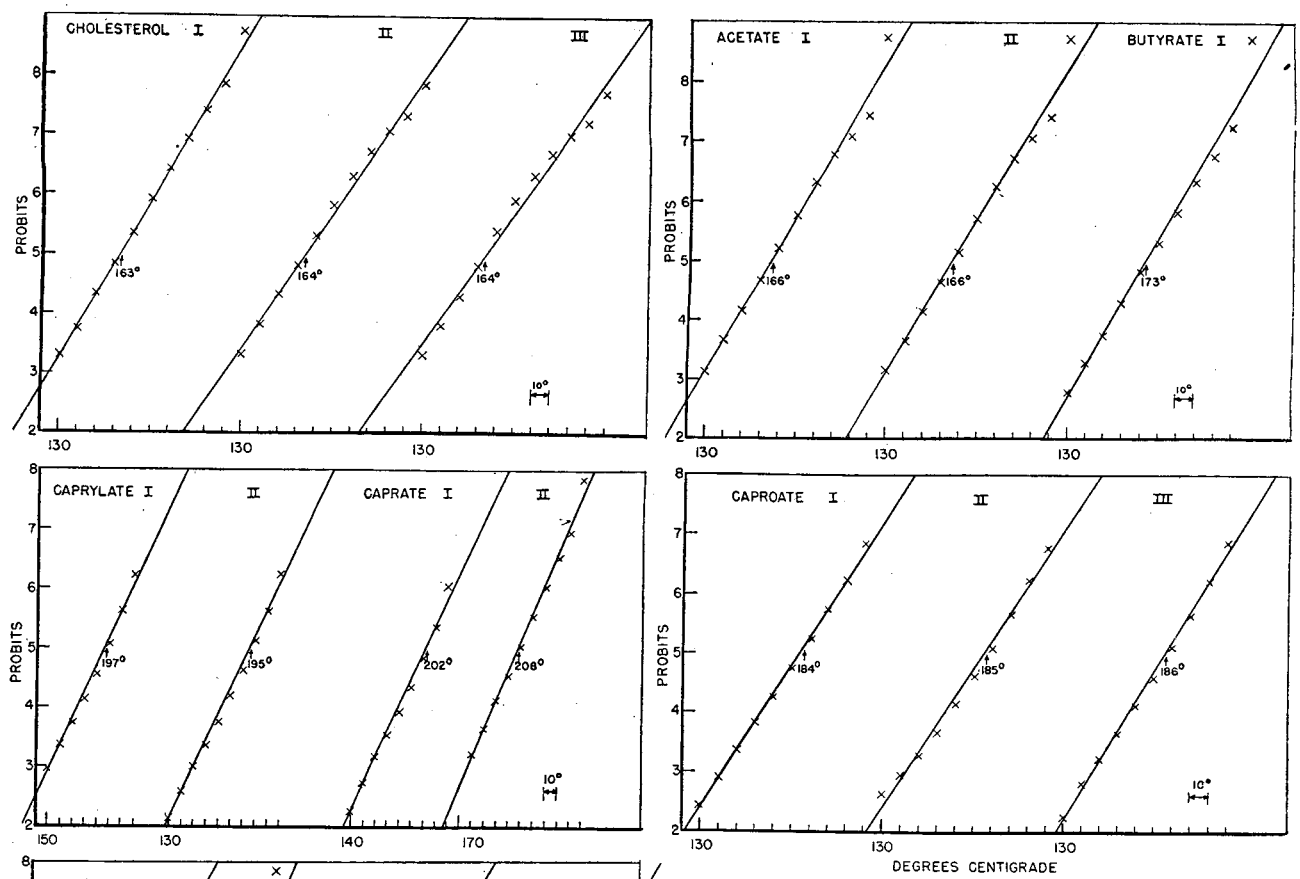

Figure 2. Graphs of Probits versus Degrees C.

Cumulative percentages distilled are plotted as probits against corresponding ° C. for each fraction. Straight lines are obtained for each distillation. Curves for cholesterol are given in upper left corner, curves for esters are shown in other sections of figure. Straight lines were obtained by method of least squares. Temperature interpolated from each line corresponding to probit 5 is the ET50 for each distillation.

The Elimination Maximum. The elimination maximum for each distillation was obtained by plotting the sterol content of each fraction against the temperature at which the fraction was obtained: actual elimination curves obtained for each ester and for cholesterol are shown in Figure 1. In practice, the determination of this reference point for each compound involves uncertainties, because the maximum of the curve must be judged by eye from the ascending and descending portions of the curve. Variations in the film thickness on the evaporator at different temperatures also markedly influence the elimination maximum. To overcome these uncertainties, a reference temperature point was determined at which 50% of the sterol had passed into the distillate. Because this point is not necessarily related to the maximum of the curve, the symbol ET50 (elimination temperature 50) is proposed for this temperature. Table II shows the data from which the elimination maxima and ET50's for the distillation of cholesterol caproate III were derived.

Calculation of Elimination Temperature 50. The elimination curves were first converted into ogives which show a characteristic S form when cumulative percentages are plotted against the distillation temperatures. By converting the cumulative percentages to a linear scale (probability transformation), a series of straight lines is obtained (Figure 2). A table for converting percentages to a linear scale is given in Table III. The excellent approximation of the data to straight lines may also be made the basis for a rapid graphical estimation of the elimination temperature 50. Since probit 5 corresponds to the mean of the normal curve (the 50% distilled point), an approximate elimination temperature 50 can be interpolated from a line fitted by eye. The experimental points appear to fall with considerable regularity on a straight line; therefore, the interpolation procedure has a corresponding ease and accuracy (*3, 4, 6, 10*).

Mathematical treatment of the data by the method of least squares (*13*), gives an equation for the line which can be solved precisely for the elimination temperature 50 point.

Table II. Cholesterol Caproate III Elimination Maximum Data

(Total dye, 5.0 ml. of 300 mg. % solution. Total sterol, 300 mg.)

Temp. ° C.	Sample Weight Mg.	Sterol Mg.	Dye Ml.	Total Sterol %	Cumulative Sterol %	Sterol, Probits
130	4.53	0.9	0.016	0.3	0.3	2.2
140	4.25	3.3	0.036	1.1	1.4	2.8
150	4.45	7.6	0.110	2.5	3.9	3.2
160	3.32	15.3	0.342	5.0	8.9	3.6
170	3.29	30.0	0.415	9.8	18.7	4.1
180	3.35	48.1	0.659	15.7	34.4	4.6
190	3.39	60.8	0.822	19.9	54.3	5.1
200	3.37	61.6	0.876	20.1	74.4	5.6
210	3.27	44.5	0.763	14.5	88.9	6.2
220	3.26	24.7	0.516	8.1	97.0	6.9
230	3.11	9.5	0.249	3.1	100.1	8.7

Table III. Table for Converting Percentages to a Linear Scale for a Normal Distribution

[(Mean set at 5.000 to avoid negative values.) In the body of the table is given the probit corresponding to each percentage listed along the left edge and top]

	0	1	2	3	4	5	6	7	8	9
0	...	2.674	2.946	3.119	3.249	3.355	3.445	3.524	3.595	3.659
10	3.718	3.773	3.825	3.874	3.920	3.964	4.006	4.046	4.085	4.122
20	4.158	4.194	4.228	4.261	4.294	4.326	4.357	4.387	4.417	4.447
30	4.476	4.504	4.532	4.560	4.587	4.615	4.642	4.668	4.695	4.721
40	4.747	4.773	4.798	4.824	4.849	4.874	4.900	4.925	4.950	4.975
50	5.000	5.025	5.050	5.075	5.100	5.126	5.151	5.176	5.202	5.227
60	5.253	5.279	5.305	5.332	5.358	5.385	5.413	5.440	5.468	5.496
70	5.524	5.553	5.583	5.613	5.643	5.674	5.706	5.739	5.772	5.806
80	5.842	5.878	5.915	5.954	5.994	6.036	6.080	6.126	6.175	6.227
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
90	6.282	6.287	6.293	6.299	6.305	6.311	6.317	6.323	6.329	6.335
91	6.341	6.347	6.353	6.359	6.366	6.372	6.379	6.385	6.392	6.398
92	6.405	6.412	6.419	6.426	6.433	6.440	6.447	6.454	6.461	6.468
93	6.476	6.483	6.491	6.498	6.506	6.514	6.522	6.530	6.538	6.546
94	6.555	6.563	6.572	6.580	6.589	6.598	6.607	6.616	6.626	6.635
95	6.645	6.655	6.665	6.675	6.685	6.695	6.706	6.717	6.728	6.739
96	6.751	6.762	6.774	6.787	6.799	6.812	6.825	6.838	6.852	6.866
97	6.881	6.896	6.911	6.927	6.943	6.960	6.977	6.995	7.014	7.033
98	7.054	7.075	7.097	7.120	7.144	7.170	7.197	7.226	7.257	7.290
99	7.326	7.366	7.409	7.457	7.512	7.576	7.652	7.748	7.878	8.090

Table IV. Summary of Distillation Data

	Elimination Maximum Sterol, ° C.	Elimination Maximum Pilot Dye, ° C.	ET50, ° C.	Equation of Line
		Cholesterol		
Cholesterol I	169	198	163	$y = 0.053x - 3.60$
Cholesterol II	169	199	164	$y = 0.045x - 2.46$
Cholesterol III	162	198	164	$y = 0.044x - 2.22$
		Cholesterol Esters		
Acetate I	171	197	166	$y = 0.052x - 3.61$
Acetate II	171	198	166	$y = 0.052x - 3.63$
Butyrate I	178	195	173	$y = 0.054x - 4.38$
Caproate I	190	...	184	$y = 0.048x - 3.90$
Caproate II	195	200	185	$y = 0.047x - 3.68$
Caproate III	196	197	186	$y = 0.050x - 4.30$
Caprylate I	204	...	197	$y = 0.046x - 4.01$
Caprylate II	204	199	195	$y = 0.044x - 3.70$
Caprate I	212	198	202	$y = 0.045x - 4.15$
Caprate II	214	196	208	$y = 0.050x - 5.34$
Laurate I	226	198	216	$y = 0.040x - 3.65$
Laurate II	226	198	215	$y = 0.050x - 5.70$
Myristate I	236	197	224	$y = 0.034x - 2.73$
Palmitate I	248	198	229	$y = 0.034x - 2.86$

DISCUSSION

A summary of the elimination maxima and ET50's obtained for each ester is shown in Table IV. The elimination temperature 50 seems slightly more accurate, as the repeated distillations of a single ester in every case (except caprate) check each other within 2°, whereas the elimination maxima in two instances differ by 6° and 7°.

The precision for this study is illustrated graphically in Figure 3. The average values for the elimination maxima and for the ET50's are plotted against the molecular weights (fatty acid chain length). A straight-line relationship on this graph might be considered ideal, as a regular difference would thus be indicated between succeeding points. Both the elimination maximum graph and the elimination temperature 50 graph closely approximate straight lines.

A line drawn by eye through the points on the elimination maximum plot shows that there is approximately a 10° interval between contiguous members of the homologous series. As the esters differ by two CH_2 groups in each instance, the similarity of this plot to the numerically comparable results obtained by Gray and Cawley (7) encourages the view that a straight-line relationship exists between the elimination maximum and molecular weight. (Cawley and Gray found that the elimination maxima of certain saturated fatty acids differed by 10° C. for each two CH_2 groups.)

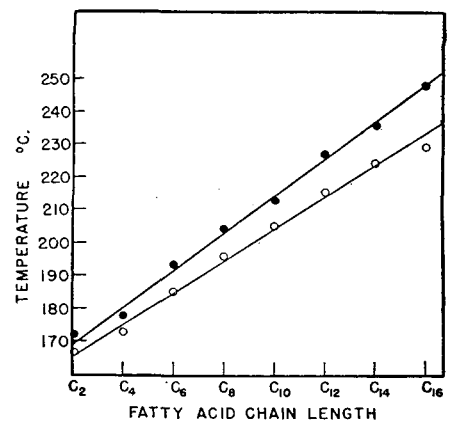

Figure 3. Fatty Acid Chain Length versus Temperature

When elimination maxima for various esters are plotted (solid circles) against ° C., a close approximation to a straight line is found. The same linear tendency is found in ET50 values (open circles). Note 10° interval between adjacent members of series.

The data obtained for the series of distillations seem in general to be of such accuracy and precision that this method could be used to identify mixed cholesterol esters if intervals of, say, 3° to 5° C., or more, existed between the maxima. In this connection, it is necessary to calculate the error of the elimination temperature 50. It was assumed that the error would be approximately the same for each compound. The average elimination temperature 50 values were computed in instances where two or more distillations had been made. The differences from the average values (14 cases) were used to compute the standard deviation of the elimination temperature 50 as 1.7° C. For two compounds—viz., cholesterol and the caproate—three distillations had been made. It was therefore possible to estimate from their means that the elimination temperature 50 plus or minus the standard error was 164° ± 1.0° and 185° ± 1.0° C., respectively.

The most important single factor in conducting a successful distillation was the constant yield oil, which should give fractions that do not vary by more than 15% in weight. In distillations where this was achieved, the results were most satisfactory.

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Determination of Dinitrotoluene in Smokeless Powder by the Titanous Chloride–Buffer Method

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When present in smokeless powder, 2,4-dinitrotoluene may be extracted with ether and determined by reduction with standard titanous chloride solution. By the use of sodium acetate or sodium citrate buffer, the reduction is caused to take place very rapidly at room temperature. This modification shortens the usual procedure.

IN THE manufacture of certain types of smokeless powders, 2,4-dinitrotoluene is added for specific purposes. During World War II, a rapid method for its determination was essential. After extraction with ether, the dinitrotoluene (DNT) was reduced to the corresponding diamine by refluxing for 5 minutes with 100% excess of standard titanous chloride solution. The excess was titrated with standard ferric alum solution, using ammonium thiocyanate indicator.

The determination of dinitrotoluene by this procedure was developed by Knecht (*2*, *3*), and adapted to the analysis of nitroglycerin-dinitrotoluene mixtures by Becker (*1*). Kolthoff and Furman (*4*) state that the reduction of nitroaromatic compounds by titanous chloride is almost instantaneous at room temperature if an alkaline buffer, such as sodium citrate, is added. This raises the pH and increases the reduction potential of the titanous ion.

The buffer method was tested by the analysis of known solutions of dinitrotoluene and ether extracts of powder samples, and found to give results agreeing closely with those found by the usual method with a saving of approximately one third in time and manipulations. Sodium acetate was slightly superior to sodium citrate as a buffer.

PREPARATION AND STANDARDIZATION OF SOLUTIONS

Titanous Chloride, 0.2 *N*. For each liter of solution, 150 ml. of 20% titanous chloride (LaMotte Chemical Products Company) were filtered through glass wool, 100 ml. of 37% hydrochloric acid were added, the solutions were mixed by means of a current of carbon dioxide, 750 ml. of water were added, and the solution was again mixed. The stock bottle was stored under an atmosphere of carbon dioxide supplied by a Kipp generator, as illustrated by Scott (*5*).

Several inorganic standards, such as ferrous ammonium sulfate, iron wire, Sibley iron ore, and potassium dichromate (National Bureau of Standards, Sample 136) are available for standardizing titanous chloride solution. The use of potassium dichromate by the following procedure is recommended:

Pass a current of carbon dioxide into a 300-ml. flask equipped with an inlet tube for 5 minutes. Add 25 ml. of 0.2 *N* potassium dichromate solution from a buret, followed by 50 ml. of 10% sulfuric acid solution. Titrate with titanous chloride solution, adding 3 drops of a 0.5% aqueous solution of sodium diphenylbenzidine sulfonate near the end point. The indicator color change is from a brownish purple, to purple, to a distinct light blue.

Ferric Ammonium Sulfate, 0.15 *N*. Use 75 grams of ferric alum and 25 ml. of 95% sulfuric acid for each liter of solution. Standardize the solution by cross titration with the standard titanous chloride solution, using 5 ml. of 20% ammonium thiocyanate as the indicator.

PROCEDURE

Displace the air in a titration flask by passing a current of carbon dioxide into it for 5 minutes. Dissolve the dried ether extract of the power sample in glacial acetic acid, and make up to volume in a 250-ml. flask. Transfer a 25-ml. portion of this solution to the titration flask. Add 30 ml. of 20% sodium acetate solution, followed by an accurately measured excess of 0.2 *N* titanous chloride solution (2.5 to 3.0 ml. for every 50 mg. of dinitrotoluene), and swirl for about 15 seconds. Add 25 ml. of 15% hydrochloric acid and titrate with the 0.15 *N* ferric alum solution, adding 5 ml. of 20% ammonium thiocyanate solution near the end point. Make blank determinations for this procedure to measure reducible impurities in the reagents. One milliliter of 1 *N* titanous chloride solution is equivalent to 0.01517 gram of dinitrotoluene.

EXPERIMENTAL

The buffering action of both sodium citrate and sodium acetate was tested. The pH of a 20% solution of each buffer was ap-

Table I. Dinitrotoluene in Three Smokeless Powder Samples

	20% Sodium Acetate		20% Sodium Citrate	
Sample	Buffer added, Ml.	DNT found, %	Buffer added, Ml.	DNT found, %
1	15	6.72	20	8.42
	20	9.18	30	9.88
	25	9.94	40	9.98
	30	9.94	50	9.94
2	15	7.64	20	7.12
	20	9.31	30	9.78
	25	9.89	40	9.91
	30	10.02	50	9.81
3	20	7.23	20	7.38
	25	7.34	30	7.31
	30	7.37	40	7.41

Table II. Determination of Known Amounts of Dinitrotoluene

	DNT Found			
	Usual Method		Sodium Acetate–Buffer Method	
DNT Present, Mg.	Mg.	%	Mg.	%
50	49.55	99.1	49.75	99.5
50	49.50	99.0	49.50	99.0
50	49.65	99.6	49.70	99.4
	Av.	99.2		99.3

Table III. Determination of Dinitrotoluene in Smokeless Powders

Sample	Usual Method, %	Sodium Acetate–Buffer Method %	Difference, %
1	9.88	9.85	−0.03
2	9.93	9.86	−0.07
3	6 69	6.63	−0.06
4	6.77	6.82	+0.05
5	6.97	7.00	+0.03
6	6.85	6.76	−0.09
7	9.90	9.87	−0.03
8	10.25	10.29	+0.04
9	9.88	9.89	+0.01
10	6.57	6.53	−0.04
11	6.79	6.74	−0.05
12	6.88	6.89	+0.01

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proximately the same. On addition to an acetic acid solution of dinitrotoluene containing an excess of titanous chloride, however, the sodium acetate raised the pH to 2.7, while the sodium citrate raised it only to 0.9. This was also shown by testing the two buffers on the ether extracts of two samples of smokeless powder containing dinitrotoluene. In Table I, it is shown that 30 ml. of sodium acetate give the same results as 40 ml. of sodium citrate. Accordingly, the former was selected as the better buffer and used in subsequent work.

A comparison was made between the buffer method and the regular boiling method, using a sample of dinitrotoluene and the ether extracts from 12 samples of smokeless powder (Tables II and III).

CONCLUSION

The use of sodium acetate as a buffer permits the reduction of dinitrotoluene to be made at room temperature and thereby shortens the usual procedure. Sodium acetate is shown to be slightly preferable to sodium citrate as a buffer. Results by the modified method agree closely with those obtained by the usual procedure.

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Determination of Nitro Nitrogen in Nitroguanidine and Cyclotrimethylenetrinitramine

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The nitro group in nitroguanidine may be reduced by boiling it with standard titanous chloride solution for 15 minutes. Although the nitro groups in cyclotrimethylenetrinitramine (RDX) are more resistant to reduction, they may by reduced by adding both titanous chloride and ferrous chloride and boiling for 30 minutes.

IN CONNECTION with the analysis of new explosive compositions during World War II, methods were needed for the determination of nitroguanidine, $O_2N.NH.C(:NH)NH_2$, and of cyclotrimethylenetrinitramine, $H_2C.N.NO_2CH_2N.NO_2CH_2N.NO_2$, popularly known as RDX. The former is a mononitro aliphatic compound with explosive properties similar to those of trinitrotoluene. Cyclotrimethylenetrinitramine is a heterocyclic, trinitro aliphatic compound, also known as cyclonite and Hexogen. It is more brisant than trinitrotoluene and is one of the most powerful of modern high explosives (*1*).

According to Bernthsen and Sudborough (*3*), nitroguanidine is easily reduced to aminoguanidine. The use of standard titanous chloride for the reduction of nitro nitrogen in explosives is described by Knecht and Hibbert (*9*), Callan and Henderson (*5*), English (*8*), and Becker (*2*). However, little has been published regarding the reduction of nitramines, where the nitro group is attached to a nitrogen atom.

When the regular 5-minute boiling method was first applied to nitroguanidine erratic results were obtained. On longer boiling, hydrolysis of the titanous chloride to yield the hydroxide occurred. However, after the acidity was increased and the solution boiled for 15 minutes, good results were obtained; one mole of nitroguanidine required 4 moles of titanous chloride.

According to Desvergnes (*7*), RDX cannot be analyzed in the nitrometer, because less than five sixths of its total nitrogen is liberated in elementary form. Rathsburg (*11*) used titanous chloride to analyze the "nitration product of hexamethylenetetramine" (RDX), but he neither balanced the equation nor identified the reaction product. He formulated the reaction

$$C_3H_6N_3(NO_2)_3 + 12TiCl_3 \longrightarrow C_3H_6N_6$$

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As nitroguanidine with one nitro group reacted with four equivalents of titanous chloride, it seemed logical to expect that RDX, with three nitro groups, would require 12 equivalents.

An attempt to reduce RDX with titanous chloride solution succeeded only to the extent of about 60%, even on prolonged boiling. Ferrous chloride effected a negligible reduction. When both reducing agents were added to the same sample, strangely enough, the reduction was over 90% complete. By use of 300% excess of titanous chloride, 20 ml. of 0.7 *N* ferrous chloride, and a 30-minute boiling period, the reduction was made to proceed to 98 to 99% of the theoretical. In view of these results, it seems likely that Rathsburg used titanous chloride that contained ferrous iron as an impurity.

As this article was being written for publication, Cottrell, MacInnes, and Patterson (*6*) described a procedure in which nitroguanidine was dissolved in concentrated sulfuric acid and titrated with ferrous sulfate solution. Their method has not been tested in this laboratory.

PURITY OF NITROGUANIDINE BASED ON NITRO NITROGEN CONTENT

The preparation and standardization of the titanous chloride and ferric alum solutions have been described (*4*).

Weigh accurately 0.09 to 0.11 gram of dry sample into a 300-ml. flask provided with an inlet tube for carbon dioxide. Dissolve the sample in 50 ml. of 1 to 1 hydrochloric acid and sweep out the air. Add 50 ml. of 0.2 *N* titanous chloride (about a 150% excess) and reflux the solution for 15 minutes. Titrate the excess titanous chloride with 0.15 *N* ferric alum solution, adding 5 ml. of 20% ammonium thiocyanate near the end point. Make blank determinations to measure reducible impurities in the reagents and apply this correction value in calculating the result. One

Table I. Reduction of Nitroguanidine with Titanous Chloride

Nitroguanidine *Gram*	Excess Titanous Chloride %	Time of Reflux *Min.*	Equivalents of Titanous Chloride Consumed per Mole of Nitroguanidine	Purity of Nitroguanidine %
0.0841	230	10	3.98	99.6
0.0905	206	10	3.99	99.7
0.1407	100	5	3.86	96.4
0.1476	88	5	3.90	97.6
0.1289	115	15	4.00	99.9
0.1320	110	15	3.97	99.3
0.0900	208	15	4.01	100.2
0.0909	205	15	4.01	100.2

milliliter of 1 *N* titanous chloride is equivalent to 0.02602 gram of nitroguanidine.

A specimen of pure nitroguanidine (recrystallized from water and dried) was analyzed by the foregoing procedure with the results shown in Table I.

PURITY OF RDX BASED ON NITRO NITROGEN CONTENT

In addition to standard titanous chloride and ferric alum solutions, 0.7 *N* ferrous chloride is required.

Weigh accurately 0.09 to 0.11 gram of dry sample into a 300-ml. flask provided with inlet tube for carbon dioxide. Dissolve the sample in 25 ml. of glacial acetic acid and sweep out the air. Add 20 ml. of 0.7 *N* ferrous chloride and 115 ml. of 0.2 *N* titanous chloride. The latter amounts to about 300% excess. Add 25 ml. of 37% hydrochloric acid and reflux the solution for 30 minutes. Titrate the excess titanous chloride with 0.15 *N* ferric alum solution, adding 5 ml. of 20% ammonium thiocyanate near the end point. Make blank determinations to determine reducible impurities in the reagents and apply a correction value in calculating the results. One milliliter of 1 *N* titanous chloride is equivalent to 0.01851 gram of RDX.

The foregoing procedure was used to determine the purity of a plant sample of RDX, with the results shown in Table II.

Table II. Reduction of RDX by Titanous Chloride Alone and by a Mixture of Titanous and Ferrous Chlorides

Weight of RDX *Gram*	Excess $TiCl_3$ Present %	0.7 *N* $FeCl_2$ Added *Ml.*	Time of Reflux *Min.*	Eequivalents of $TiCl_3$ Consumed per Mole of RDX	Purity of RDX %
0.1537	136	0	20	7.00	58.3
0.1443	150	0	20	7.00	58.3
0.1400	151	0	20	7.19	59.9
0.1300	172	0	20	7.39	61.6
0.1500	60	20	5	10.50	87.5
0.1480	52	20	10	11.22	93.5
0.1566	46	20	10	11.09	92.4
0.1500	53	20	15	11.04	92.0
0.1500	53	20	20	11.04	92.0
0.1500	51	20	20	11.14	92.8
0.1086	176	20	20	11.66	97.2
0.1055	209	20	30	11.68	97.3
0.1019	257	20	30	11.86	98.8
0.1000	325	20	30	11.78	98.2
0.1023	315	20	30	11.93	99.4
0.1000	306	20	30	11.88	99.0
0.1028	314	20	30	11.90	99.2
0.1130	331	20	30	11.96	99.7
0.1017	320	22	30	11.84	98.7
0.1017	322	30	30	11.81	98.4

SUMMARY

Correct conditions for the quantitative reduction of the nitro groups in nitroguanidine and cyclotrimethylenetrinitramine (RDX) were developed; four equivalents of titanous chloride are required for the reduction of each nitro group present. In the case of RDX, ferrous chloride as well as titanous chloride must be used to effect complete reduction. Although the reactions appear to be stoichiometric, the reduction products were not isolated and equations were not written.

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Determination of Potassium Perchlorate in Smokeless Powder

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DURING World War II, potassium perchlorate was added to certain types of smokeless powder for specific purposes, and a rapid method for its determination in powder was needed.

Two methods giving satisfactory results have been developed. The first, or explosion-Volhard method, consists in decomposing a ground powder sample by explosion in a Parr stainless-steel calorimeter bomb. The explosion quantitatively reduces the perchlorate to chloride, which may best be determined by Caldwell and Moyer's (*2*) nitrobenzene modification of the Volhard method (*7*). The second, or nitric acid–titanous chloride method, is longer and less accurate than the explosion-Volhard method, but it avoids need for a Parr bomb. It involves decomposition of the powder sample with nitric acid, subsequent removal of nitric acid and carbon black (if present), and determination of the potassium perchlorate by reduction with titanous chloride.

The analysis of perchlorates by reduction with titanous chloride was first suggested by Knecht (*3*) and used by Knecht and Hibbert (*4*), who titrated potassium perchlorate in strong sulfuric acid solution, after adding oxalic acid to facilitate the reduction. Rothmund and Burgstaller (*5*), however, did not consider the presence of oxalic acid necessary. Vil'yamovich (*6*) succeeded in completely reducing potassium perchlorate in strongly acid (sulfuric and hydrochloric) solution by using approximately 400%

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When present in smokeless powder, potassium perchlorate may be determined by explosion of the sample in a calorimeter bomb and use of the Volhard method for chloride, or by decomposition of the sample with nitric acid, followed by reduction of the perchlorate with standard titanous chloride solution. The first method is preferable, if the equipment is available, because it is more accurate and requires less time

excess of titanous sulfate and refluxing for 2 hours. The authors have found that while at least a 33% by weight acid solution must be maintained, potassium perchlorate may be completely reduced by refluxing for 5 minutes after the addition of only 100% excess of titanous chloride.

PROCEDURE

Explosion-Volhard Method. To decompose the sample, a stainless-steel bomb was used, manufactured by the Parr Instrument Company, designated Model B, oxygen, single valve. During the explosion of a sample, the bomb should be placed behind a substantial shield or barricade, for safety.

Add 25 ml. of water to the bomb, weigh a ground and blended 5-gram sample into the cup, and place the cup in the bomb. Attach the fuse wire, screw on the cover, and make the electrical connections. Explode the sample at atmospheric pressure and allow the bomb to cool for 5 minutes in the air before releasing the gas. Wash the bomb thoroughly with several portions of water, using a total of 200 ml.; take particular care to wash through the valve and the inside of the bomb top and the posts. Evaporate the washings to about 80 ml. and dilute them to 100 ml. in a volumetric flask. Determine the chloride by the modified Volhard method, using a 10-ml. aliquot, 10 ml. of 35% nitric acid, 2 ml. of nitrobenzene, and 0.05 *N* silver nitrate and 0.05 *N* potassium thiocyanate solutions.

Nitric Acid—Titanous Chloride Method. The preparation and standardization of the titanous chloride and ferric alum solutions have been described (*1*).

Table I. Analysis of Saltless Powder Samples Containing Added Potassium Perchlorate

Potassium Perchlorate Added, *Gram*	Potassium Perchlorate Recovered, *Gram*	Recovery, %
	Explosion-Volhard Method	
0.4000	0.3988	99.7
0.4065	0.4028	99.1
0.4075	0.4042	99.2
0.4028	0.3984	98.9
0.4042	0.4038	99.9
0.4000	0.4000	100.0
0.4043	0.4059	100.4
0.7900	0.7837	99.2
0.4650	0.4608	99.1
0.4000	0.3988	99.7
	Nitric Acid–Titanous Chloride Method	
0.3905	0.3968	101.6
0.3907	0.3782	96.8
0.3932	0.3943	100.3
0.3900	0.3877	99.4
0.3939	0.3993	101.4
0.3900	0.3757	96.3
0.3900	0.3841	98.5
0.3907	0.3917	100.3
0.3908	0.3793	97.1

Table II. Analytical Results on Plant Samples of Smokeless Powder Formulated to Contain 7.8% Potassium Perchlorate

Sample	Potassium Perchlorate Found: Explosion-Volhard method, %	Potassium Perchlorate Found: Nitric acid–titanous chloride method, %
1	7.63	7.39
2	7.45	7.04
3	7.68	7.81
4	7.57	7.50
5	7.61	7.70
6	7.58	8.06
7	7.73	7.58

Weigh a 5-gram sample of powder into a 150-ml. beaker and add 70 ml. of 70% nitric acid. Cover with a watch glass and digest for 2 hours or until red fumes are no longer copiously evolved.

Remove the cover glass, evaporate to about 10 ml., add 20 ml. of glacial acetic acid, and evaporate to approximately 10 ml. Filter the solution while hot through a Gooch crucible or sintered-glass funnel. Wash the beaker with a small amount of hot water and filter this also. Transfer the filtrate and washings quantitatively back to the original beaker and evaporate to dryness on a steam bath. Add 25 ml. of water and evaporate the solution to dryness. Repeat the addition of water and its evaporation to effect the complete removal of nitric acid. Again add 25 ml. of water and heat the solution to approximately 70° C.

Filter the warm solution through a Whatman No. 41 filter paper into a 100-ml. volumetric flask. Wash both beaker and filter paper with small portions of warm water. Cool the flask to room temperature before diluting the solution to exactly 100 ml.

Add a 10-ml. aliquot to a 300-ml. flask provided with an inlet tube for carbon dioxide. Sweep out the air, add 10 ml. of 95% sulfuric acid and 100% excess of 0.2 *N* titanous chloride solution (1 ml. for every 1.7 mg. of potassium perchlorate estimated to be present in the aliquot), connect the flask to a condenser, and reflux the solution for 5 minutes. Titrate the excess titanous chloride with the 0.15 *N* ferric alum solution, adding 5 ml. of 20% ammonium thiocyanate near the end point. Make a blank determination to measure reducible impurities in the reagents. One milliliter of 1 *N* titanous chloride is equivalent to 0.01732 gram of potassium perchlorate.

RESULTS

In order to test the explosion-Volhard method, known mixtures whose composition simulated that of regular-production potassium perchlorate powders were prepared and analyzed. Their preparation was accomplished by mixing known amounts of potassium perchlorate with ground samples of a saltless smokeless powder containing nitrocellulose, nitroglycerin, centralite, and carbon black. The analytical results obtained are given in Table I, together with results found by the nitric acid–titanous chloride method on similar samples. Finally, seven regular-production lots of smokeless powder to which 7.8% potassium perchlorate had been added during manufacture were analyzed by both procedures, with the results shown in Table II.

It may be seen from Tables I and II that, although the average results found by the two methods agree reasonably well, those obtained by the explosion-Volhard method are much more concordant. The latter method has the added advantage that results can be obtained in about 2.5 hours, while the nitric acid–titanous chloride method requires about 12 hours.

ACKNOWLEDGMENT

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Determination of Phenol and *m*-Cresol in Complex Biochemical Mixtures

Application of Countercurrent Distribution Method

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Phenol can be quantitatively determined in the presence of the isomeric cresols; mixtures of *m*-cresol and phenol can be determined in complex biochemical samples. The phenolic compounds are removed from the interfering substances by distillation at pH 8.0 to 8.5, and the sum of phenol and *m*-cresol is determined by the sulfanilic acid method. Phenol is then separated from the cresols by submitting an aliquot of the distillate to a 24-plate countercurrent distribution. From the amount of phenol found in a selected series of tubes, the concentration in the original sample can be calculated. *m*-Cresol, in the presence of phenol, is determined by difference by subtracting the value for phenol from the total phenolic content of the sample. Some volatile phenolic compounds may interfere with the method.

ALTHOUGH numerous procedures have been described for the determination of phenol or phenolic compounds in simple aqueous solutions (*13*), no satisfactory method for the simultaneous determination of dilute solutions of mixtures of these compounds in biological samples is available. Such a procedure would undoubtedly have appreciable value for clinical, bacteriological, and industrial analysis where it is often desirable to determine the concentration of each component of a phenolic mixture.

Several procedures have been applied to this problem, but they have only a limited scope. Düll (*5*) and Potter and Williams (*9*) determine *o*-cresol in phenol by forming a complex with cineole and determine the complex by freezing point data. Another method (*14*) is based upon the lowering of the freezing point of phenol by cresol. A similar procedure, but one that has been found to yield more reliable results, is the cloud point method of Seaman, Norton, and Foley (*12*). These methods are, however, applicable only to simple mixtures of phenol and cresol where the only other component is water. A different approach to the problem was described by Bielenberg and Fischer (*1*), who coupled mixtures of hydroxybenzene compounds, such as phenol and cresol, with diazotized *p*-nitroaniline to form individual azo colors which were then separated chromatographically and determined colorimetrically. Miller and Urbain (*8*) report that phenol is quantitatively oxidized by chromic acid while cresols and the higher phenols such as resorcinol are unaffected. They propose a method for estimating phenol by difference before and after chromic acid oxidation. This procedure was investigated by the present authors and it was found that some cresol was oxidized as well as the phenol. A spectrographic method has also been used for determining the concentration of phenol and cresol in mixtures (*10*), but this procedure is not applicable to dilute aqueous solutions of these compounds.

Inasmuch as the original purpose of this investigation was the determination of the phenol content of bacteriological materials and media, several phenol methods were tried directly on the complex samples. No one method, however, was found satisfactory for this purpose. The solution of this phase of the problem was eventually found in the removal of the phenol from the bacteriological materials that contained the interfering substances. This separation was accomplished by distilling the phenol from the mixture after adjusting the pH to 8.0 to 8.5. At this pH the interfering substances were held back as salts while the phenol was free to distill. It was found that when 80 to 90% of the water was distilled, 99 to 100% of the phenol passed over into the distillate. After the separation of the phenol from the interfering substances, the determination could be completed by any one of the standard procedures. Although the Folin and Ciocalteu method (*6*) was initially used for the colorimetric determination of the phenol, it was not so sensitive as the diazotized sulfanilic acid method. Consequently, the latter procedure was followed, as it was desired to determine accurately microgram quantities of the phenolic compounds.

Once it was learned that phenol could be quantitatively separated from complex biochemical substances, the effect of cresol was investigated. This study showed that cresol and phenol behaved similarly and could not be easily distinguished from each other. It was eventually decided to investigate the possibility of separating phenol from cresol by means of the countercurrent distribution technique of Craig and co-workers.

For a comprehensive description of the method, apparatus required, theory involved, and some applications of the technique, it is recommended that the papers of Craig (*2, 3, 4, 7, 11, 15*) be consulted. The fundamental basis of countercurrent distribution is the partition of a soluble substance between two immiscible phases. The relative concentration of the solute in each of the phases is a function of the partition coefficient of the substance, and, under specified experimental conditions, is a constant ratio. This partition coefficient is an identifying characteristic of pure compounds and has been used to determine the heterogeneity of otherwise inseparable mixtures. This was accomplished by utilizing the fact that although compounds may be chemically similar in structure, they often may be shown to have slightly different partition coefficients when distributed between a proper choice of two immiscible solvents. This slight difference in the preferential solubility in one of the solvents can be amplified by repeating the process a sufficient number of times, so that for practical purposes a solution may be obtained in which only one component is present; the more soluble compound may be extracted, or all but the least soluble component of the mixture removed.

The Craig-machine (*3*) is a convenient apparatus for conducting this series of stepwise liquid-liquid distribution measurements without necessitating the tedious and cumbersome use of a large number of individual separatory funnel extractions. Although it was successfully utilized in the resolution and characterization of the penicillins, the isolation of antibiotic principles from *Aspergillus ustus*, and the study of the homogeneity of

antimalarials, it has received only limited use in the field of analytical chemistry. This instrument promises to be of appreciable value in the solution of analytical problems in which it is difficult to eliminate the error due to interfering substances. That it is also applicable to simultaneous determination of a mixture of chemically similar compounds was shown by Sato, Barry, and Craig (*11*), who succeeded in separating and quantitatively estimating within 2 to 3% the volatile normal fatty acids (C_2 to C_5) in a mixture. The procedure was equally successful in the present problem of resolving the components of the phenol-cresol mixture sufficiently to permit quantitative determination of each constituent. This was ultimately effected by subjecting the mixture to a 24-plate distribution between carbon tetrachloride and potassium acid phthalate buffer of pH 5.0. From the amount of phenol detected in one of the phases of a selected series of tubes, the quantity of phenol present in the original sample was calculated. *m*-Cresol was found by difference by subtracting the value for phenol from the total amount of phenol plus *m*-cresol in the initial mixture.

APPARATUS AND REAGENTS

Stock standard solutions of phenol and *m*-cresol were prepared by diluting Merck's pure analytical reagent grade phenol and synthetic *m*-cresol (prepared from *m*-toluidine), boiling point 201° C., with distilled water to give a concentration of 1.000 mg. per ml. These solutions were diluted further to 5 micrograms per ml. for use in preparing the standard curve for the colorimetric determination of phenol and cresol.

The sulfanilic acid reagent was prepared according to the procedure described by Snell (*13*): Mix 4.5 grams of sulfanilic acid with 45 ml. of concentrated hydrochloric acid and dilute to 500 ml. with water. In another 500-ml. volumetric flask dissolve 25 grams of sodium nitrite in water and dilute to the mark. Cool 3.00 ml. of the sulfanilic acid solution in a 100-ml. volumetric flask in an ice-water bath, add 3.00 ml. of the sodium nitrite solution, cool for 5 minutes, and then add 12.0 ml. of the sodium nitrite solution. Make up to a volume of 100 ml. with distilled water and keep the final reagent solution cold. This reagent can be used after 15 minutes, and is prepared fresh daily.

The two immiscible solvents used for the countercurrent distribution of the phenol and *m*-cresol were Merck's reagent grade carbon tetrachloride and 0.1 *M* potassium acid phthalate buffer of pH 5.0.

For the separation of the phenolic compounds from the interfering substances in the sample, a simple Kjeldahl distillation unit, consisting of a 100-ml. Kjeldahl flask, a water-trap, and a condenser was used.

The Craig apparatus was a 24-tube, stainless steel unit, which was purchased from Otto Post, Maspeth, N. Y., and had a capacity of 8 ml. for each tube of the lower section.

A Coleman Universal spectrophotometer, Model XI, was utilized for colorimetric determination of the phenol. Matched cuvettes of 18-mm. diameter were used for measuring the intensity of light transmitted.

ANALYTICAL PROCEDURE

Distillation of Phenolic Mixture. In the present work it was found convenient to employ a sample containing approximately 1 mg. of phenol and 1 mg. of *m*-cresol. Samples with a higher concentration of the phenolic compounds can also be submitted to the distillation procedure if smaller aliquots of the distillate are subsequently used for the countercurrent separation.

Approximately 25 ml. of 0.1 *M* sodium bicarbonate solution at pH 8.1 were added to the sample in a 100-ml. Kjeldahl flask and the total volume was diluted to about 60 ml. with distilled water. The flask was gently heated and the distillate was collected in a 200-ml. volumetric flask immersed in an ice bath. The distillation was continued until about 2 to 5 ml. of solution remained in the Kjeldahl flask. The end of the condenser was rinsed with water and the distillate diluted to the 200-ml. volume.

Separation of Phenol from *m*-Cresol. The carbon tetrachloride which was used as the heavy phase in the distribution apparatus was saturated with the aqueous buffer by shaking 250 ml. with 250 ml. of the phthalate buffer. The lower half of the apparatus is filled with the carbon tetrachloride in the manner prescribed by Craig, which consists of adjusting the upper section so that each hole covers half of the corresponding hole in the lower section. In this manner, all the cells of the lower section can be filled in one operation by pouring solvent into a single tube. As the tubes are interconnected, the liquid will overflow into all the holes. To ensure the presence of equal volumes of solvent in all tubes, the upper section is removed and the remaining sections are filled so that the meniscus is level with the surface of the bottom section. The top section is then replaced and set so that the numbered tubes of both sections correspond, and exactly 8 ml. of the buffer (previously saturated with carbon tetrachloride) are added by means of a buret to all the tubes except the zero tube. An aliquot of the sample containing from 100 to 150 micrograms of both phenol and *m*-cresol is placed in the upper part of the zero tube and sufficient buffer to bring the total volume of the upper layer to 8 ml. is added.

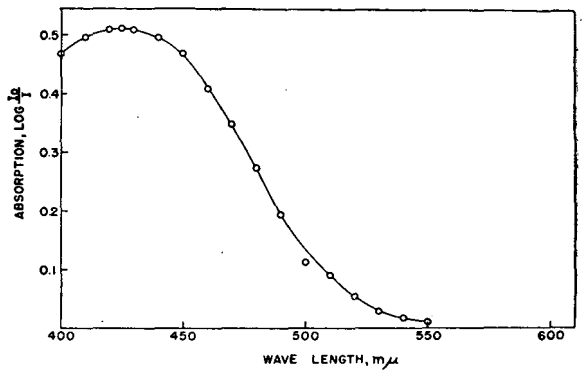

Figure 1. Absorption Spectrum of Colored Product Resulting from Reaction of Diazotized Sulfanilic Acid with Phenol and *m*-Cresol

The apparatus is assembled, with all the parts in place and water-tight. With the holes arranged in the starting position, the instrument is rotated 25 times at a speed which will ensure proper mixing of the phases. This can be observed in the glass indicator tube that is attached to the metal body of the machine and contains 8 ml. of both liquids. When equilibrium is attained, the layers are allowed to separate, and the upper section is carefully moved so that the upper portion of the zero tube is now superimposed on the lower half of the No. 1 tube. The same series of manipulations is repeated for all 24 tubes. Upon completion of the partition operations, the pressure plate is removed and a 4-ml. aliquot of the water layer in each of the tubes is pipetted out into a test tube in preparation for the colorimetric determination of the phenolic content of the individual tubes. In the actual determination of an unknown sample, the analysis of only three or four suitably selected tubes is necessary.

Colorimetric Determination. To the aliquot from the countercurrent distribution treatment are added 1 ml. of a 20% sodium carbonate solution and water to make the total volume 8 ml. Two milliliters of the cold sulfanilic acid reagent are added to the mixture, which is shaken for 1 to 2 minutes, and the per cent transmittance is read in the spectrophotometer against a blank of the reagents at a wave length of 425 mμ. The amount of total phenols in the aliquot sample is calculated (as phenol) from a standard curve constructed from a series of known amounts of phenol in the range of 1 to 20 micrograms.

CALCULATIONS

The calculations for the amount of phenol in the aliquot sample subjected to the countercurrent distribution are based on the theoretical equations for the partition of a substance between two immiscible solvents. These equations have been discussed by Williamson and Craig (*15*), and involve a consideration of the partition coefficient of phenol in the system selected and the number of single extractions used to effect the desired separation. It can be shown experimentally that the solute in such a system is distributed between the two phases in accordance with the binomial expansion theorem. Consequently, by determining the amount of solute in any particular tube, it is relatively simple to calculate the concentration in the original sample. For reasons mentioned below, it was found more accurate to estimate the initial phenol concentration from the phenol content of the aqueous layer only rather than from the total amount of the entire

tube. Thus, merely by determining the weight of phenol in the water layer of three or four tubes, it is possible to calculate the concentration of phenol in the original sample.

The expression relating the partition coefficient with the fraction of the original substance present in any tube, r, in a distribution of n transfers or plates is given by Craig as:

$$T_{n,r} = \left(\frac{n!}{r!\,(n-r)!}\right)\left(\frac{1}{k+1}\right)^{n}(k)^{r} \qquad (1)$$

where $T_{n,r}$ = fraction of original substance in tube r, n = total number of transfers, and k = partition coefficient.

The method used in this study for the determination of the partition coefficient of the solute utilized the condition that the ratio of the concentrations in any two adjacent tubes is a specific function of the partition coefficient. This relationship is given by the equation

$$k = \frac{1}{F} \times \frac{T_r}{T_{r-1}}$$

where F is the factor for relating T_r to T_{r-1}, and T_r and T_{r-1} are amount of solute in the tube r and $r - 1$. The factor, F, is, in turn, calculated by the formula,

$$F = \frac{n+1-r}{r}$$

Thus, by selecting any two adjacent appropriate tubes (those tubes corresponding to the peak of the distribution curve) the partition coefficient can be readily calculated from the amount of phenol present in the aqueous layer. For example, in one experiment involving a 24-plate transfer of phenol and *m*-cresol, tube 9 contained 15.2 micrograms of phenol in the upper water layer and tube 8 contained 16.2 micrograms of phenol. For tube 9, therefore, $F = \frac{24+1-9}{9} = \frac{16}{9}$.

The partition coefficient, $k = \frac{9}{16} \times \frac{15.25}{16.20} = 0.529$.

By substituting in Equation 1 the correct value for k and expanding the expression to find the rth term, the fraction of the original phenol present in any tube can be obtained. For example, using the same data as in the preceding paragraph for tube 9, $T_{n,r} = (1.30 \times 10^6) \times \left(\frac{1}{1+0.529}\right)^{24} \times (0.529)^9 = (1.30 \times 10^6) \times (3.88 \times 10^{-5}) \times (3.25 \times 10^{-3}) = 0.164$. By dividing this fraction into the value for the phenol content found in the aqueous layer of tube 9, the phenol present in the sum of all the water layers will be obtained—e.g., $\frac{15.25}{0.164} = 93.0$ micrograms. Inasmuch as this figure represents only the quantity found in one phase—i.e., the water layers—it is necessary to calculate from the partition coefficient the amount present in the other solvent layer. Because, in the present instance, the partition coefficient is 0.529, the amount of phenol present in the carbon tetrachloride is 0.529 × 93.0 = 49.3 micrograms. The sum of these two values (93.0 + 49.3 = 142.3) gives a quantitative measure of the phenolic content of the aliquot sample submitted to the countercurrent distribution separation.

Table I. Recovery of Added Phenol

Phenol Added	Phenol Found	Phenol Recovered
Mg.	*Mg.*	%
1000	1000	100
800	790	98.8
500	500	100
200	200	100
100	100	100
2.00	1.98	99.0
1.00	1.01	101
0.00	0.00	...

DISTILLATION AND DETERMINATION OF PHENOLIC MIXTURES

Using the conditions described under Procedure, samples of complex biochemical mixtures containing various amounts of phenol were used to investigate the reliability of the separation from the interfering substances. The results of this work showed that recoveries of almost 100% phenol can be achieved (Table I). If the pH of the bacteriological sample was not previously adjusted to 8.0 to 8.5, errors resulting from interfering substances amounted to as much as 20% relative. A similar series of experiments with cresol gave essentially the same results. In an attempt to separate cresol and phenol by distillation at different pH values mixtures of the two were distilled from aqueous solutions at various pH's ranging from 8 to 10. Table II shows the effect of pH on the recovery of these compounds by distillation. The values obtained in these experiments make it clear that distillation at a controlled pH is unsatisfactory as a means of separating phenol from the cresols.

Table II. Effect of pH on Distillation of Phenol and *m*-Cresol

pH	Per Cent Recovery	
	Phenol	*m*-Cresol
8.0	100	100
8.5	100	101
9.0	94.0	96.2
9.5	80.6	89.7
10.0	61.0	74.4

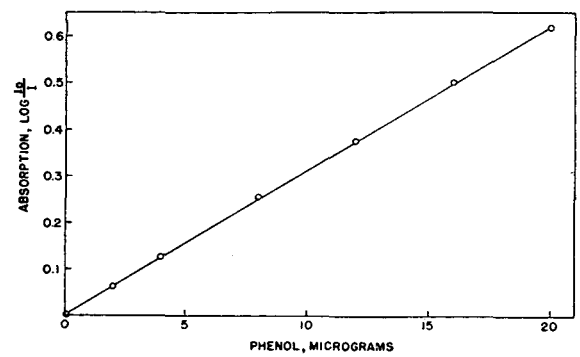

Figure 2. Effect of Concentration of Phenol on Intensity of Color

A partial investigation of the specificity of the distillation procedure was also undertaken. As the only substances that would interfere with the method as described are volatile phenolic compounds, higher phenols were subjected to the same treatment. It was found that orcinol, resorcinol, phloroglucinol, and hydroquinone were not distilled under the conditions used and did not affect the accuracy of the method; 2-hydroxy-1,4-dimethyl phenol, however, did interfere. No attempt was made to adapt the present procedure to mixtures containing this compound.

The diazotized sulfanilic acid method for determining the phenol and *m*-cresol was found very satisfactory. As no curve of the absorption spectrum of the colored product was available, this information was determined. On the basis of the results illustrated in Figure 1, it was considered advisable to select 425 mμ as the wave length for all subsequent colorimetric measurements. The relationship between the concentration of phenol and the log of per cent transmittance at this wave length is shown in Figure 2. Except for differences in molecular weight, *m*-cresol was found to give the same transmittance as phenol. Therefore, in the determination of the total phenolic content of a sample, only one standard curve for phenol is required; *m*-cresol, obtained ultimately by difference, will be computed in terms of phenol and must be corrected by multiplying by the ratio of the molecular weights. The intensity of the color developed in an aqueous medium was found to be entirely different from that found in carbon tetrachloride. For this reason, all determinations were

Table III. Determination of Phenol in Known Samples

Run No.	Phenol Present in Original Sample	Tube No.	Phenol Found in Buffer Layer	Partition Coefficient	Phenol Calcd. in Original Sample	Recovery
	Mg.		*Mg.*		*Mg.*	%
1	140	0	2.2			
		1	10.7			
		2	21.5	0.447	139.5	
		3	25.8	0.449	140.9	
		4	20.1	0.445	140.0	
		5	10.5			
		6	3.1			
		7	0			
		8	0			
		9	0			
			Av.	0.447	140.1	100.1
2	140	0	2.6			
		1	10.9			
		2	22.4	0.456	148.0	
		3	27.0	0.446	148.0	
		4	21.3	0.451	148.2	
		5	11.6			
		6	3.4			
		7	0			
		8	0			
		9	0			
			Av.	0.451	148.0	105.8
			Av., runs 1 and 2		144.8	102.9

Table IV. Separation and Determination of Phenol and *m*-Cresol

Sample No.	Phenol Present	*m*-Cresol Present	Total Phenols Found[a]	Total Phenol Content Calculated from Tube No. 6	No. 7	No. 8	No. 9	Av.	Error	*m*-Cresol (by Difference)	Error
	γ	γ	γ	γ	γ	γ	γ	γ	%	γ	%
1	140.0	141.0	262.0	139.9	136.6	150.0	145.6	143.0	+2.1	134.0	−5.0
2	140.0	141.0	263.6	139.2	137.9	138.3	142.9	139.6	−0.3	139.8	−0.9
3	105.0	95.0	185.8	104.3	104.5	105.8	111.2	106.4	+1.4	89.5	−5.8

[a] Phenol and *m*-cresol determined in terms of phenol.

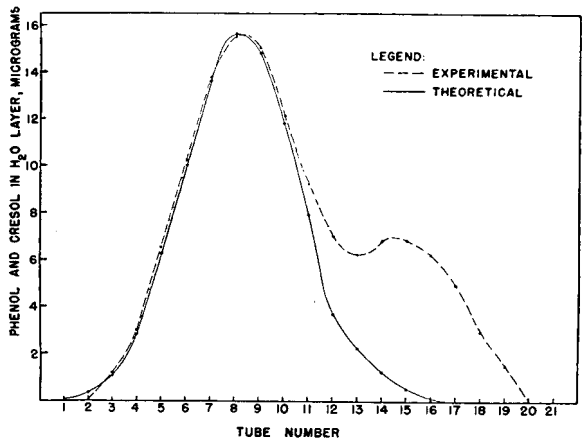

Figure 3. 24-Plate Countercurrent Transfer Distribution of Phenol and *m*-Cresol between Carbon Tetrachloride and Phthalate Buffer

140 γ each. pH 5.0. Broken line represents experimental curve for 140 γ each of phenol and *m*-cresol. Solid line is theoretical curve for phenol only

made on only the aqueous layer of the tubes and not the total contents.

COUNTERCURRENT SEPARATION

It was found that the partition coefficient of phenol is not markedly affected by the pH of the aqueous phase. Nevertheless, as a precaution, a buffer solution of sufficient buffer capacity to maintain a constant pH was used. A buffer of about pH 5 was arbitrarily selected on the basis that the partition coefficient of phenol at this pH was sufficiently different from the partition coefficient for *m*-cresol to make the separation possible, and yet was not too far removed from the optimum value of 1.

A preliminary application of the procedure to the quantitative determination of a known amount of phenol was made using a 10-plate transfer. Determination of the phenol content of the buffer layer of several tubes showed that tube 3 contained the maximum amount. The average partition coefficient was calculated for tubes 2, 3, and 4 and found to be 0.447. Using this average value as the most probable, calculations similar to those shown in the preceding section for computing the concentration of phenol in the original sample were made. The data of this and another similar experiment are shown in Table III. The initial indication of the probability of separating phenol from the cresols was the observation that the partition coefficient of *m*-cresol was significantly different from that of phenol under the conditions used. Partition coefficient measurements with *m*-cresol between the solvents selected gave an average value of 1.25. As a maximum degree of separation was desirable, the countercurrent distribution was extended to a 24-plate transfer, and applied directly to a synthetic mixture of the two compounds. Figure 3 is a graphic representation of the distribution curve obtained upon analysis for total phenols of the buffer layer of each tube. Also included in this figure is a plot for the theoretical curve of phenol calculated from the equation for the binomial expansion theorem, as indicated previously. The results of the actual determination of the phenol and *m*-cresol content of several samples of a synthetic mixture are given in Table IV. The samples were from a sludge of *B. globigii* to which known amounts of phenol and *m*-cresol were added. They were subjected to the entire procedure, including distillation and countercurrent distribution separation. Considering complex nature of the material analyzed, errors indicated in Table IV are not excessive.

Although the procedure has been described for the determination of phenol and the meta form of cresol, the phenol can be separated from and determined directly in the presence of the other isomers as well. However, since the cresol is eventually determined by difference, it would be necessary to investigate the effect of all the pure synthetic isomers on the intensity of the colorimetric measurements before mixtures of all the cresols can be accurately determined. This study has not yet been undertaken.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of J. P. Crispell in investigating the applicability of many phenol methods and of Ilse Schwab in performing some of the chemical determinations.

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Determination of Malt Sirup Density

Application of the Brabender Recording Viscometer to Sirup Densimetry

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The use of the Brabender recording viscometer for the rapid determination of malt sirup densities has been demonstrated. An extensive application of the viscometer in routine control work has shown an accuracy comparable to that obtained with the hydrometer and pycnometer. The application of this method to other types of sirup has been indicated.

CUSTOMER specifications for malt sirups often set narrow limits for the range of total solids content, and necessitate accurate determinations of sirup densities. In addition, shipping schedules often require that density data be made available in the shortest possible time. Thus, accuracy and speed are very essential for estimations of malt sirup density.

Most widely used for determining sirup density are the hydrometer, pycnometer, and refractometer procedures as outlined in the methods of analysis of the Association of Official Agricultural Chemists (*1*). The use of hydrometers, calibrated to read directly in degrees Baumé, is probably the most simple and direct method of measuring malt sirup concentration. For "free-flowing" sirups around 42° Bé. density, the hydrometer method is satisfactory, but with certain types of sirup, especially those of higher densities, the hydrometer no longer gives reliable readings. The composition of the sirup also has an effect on the accuracy of hydrometer values. As the ratio of nonsugar solids to sugar solids increases, the tendency for the hydrometer to give too high a density reading becomes greater. The time required to obtain an accurate hydrometer reading on an undiluted sirup decreases the value of the procedure as a rapid control method.

Malt sirup density may be accurately and reliably determined by means of the specific gravity as obtained with a pycnometer. However, the necessary steps of sirup weighing, dilution, tempering to a standard temperature, volume adjustment, and final weighing require considerable time.

The refractometer method is applicable only to sirups containing no undissolved solids. Refined corn sirups fall in this category, but malt sirups containing varying amounts of colloidal protein material cannot be accurately analyzed for solids content by a method dependent upon light transmittance.

Thus, the methods most widely used for determinations of sirup density do not have the required combination of rapidity and accuracy. The possible use of some other physical property of sirups to determine solids was considered. The most obvious characteristic which might be correlated with density is the consistency or viscosity.

Bingham and Jackson (*2*) showed the effect of solids content and temperature on the viscosity of pure sucrose solutions. Chatoway (*3*), followed by Oppen and Schuette (*6*) developed quantitative relationships between the density and viscosity of honey, and demonstrated that the moisture content of honey could be calculated from viscosity measurements. Miller and Mench (*5*) studied the viscosity-density correlation for corn sirups, and showed the effect of composition by working with sirups of varying dextrose equivalents.

The above workers used very precise instruments and techniques to obtain their viscosity values, as the expression of viscosities in absolute units required extremely accurate control of all variables. Although such procedures are necessary for fundamental research, they are not particularly adaptable to routine control work. Furthermore, the very nature of sirupy material makes instrument cleaning a tedious process with viscometers of the Ostwald and Hoeppler types. Consequently, a survey of other viscometers was made, with emphasis on simplicity and ease of operation. The instrument that appeared to meet the desired qualifications was a recording, rotational type of viscometer designed and developed by Wicker and Geddes (*7*) in conjunction with the Brabender Corporation, Rochelle Park, N. J., for the specific purpose of measuring paint consistency. The instrument fills the desired requirements of speed, ease of cleaning, and simplicity of operation. Consequently, the possibility of applying this viscometer to malt sirups was considered.

The operating principles of the Brabender viscometer are relatively simple. The sample container is rotated at constant speed by a revolving platform, imparting a torque to a paddle submerged in the sample. This torque is opposed by a helical spring, so that the angular displacement of the spring is a measure of the induced torque. By means of a lever arm and inking mechanism, a permanent record of the spring displacement is obtained.

The machine may be used to cover a wide range of consistencies, as there are three factors that can be varied. The speed of the platform rotation can be changed by the use of different pulley combinations, the resistance to the torque can be changed by using various sizes of spring wire, and the torque can be varied by changing the size and shape of the submerged paddle.

EXPERIMENTAL METHOD

The proper combination of speed, spring strength, and paddle design had to be determined as the first step in adapting the Brabender instrument to the measurement of malt sirup consistency. Experimental work showed that the lowest speed on the Model A instrument, 50 r.p.m., with a pin paddle as shown in Figure 1, in combination with either a light (d = 0.07 cm., 0.028 inch) or a heavy (d = 1.11 cm., 0.444 inch) spring, covers the practical range of malt sirup consistencies. The heavy spring had to be used at higher densities in order to keep the recorder pen within the limits of the chart. As two springs were used, a factor was required to convert from one spring reading to the other. This factor was obtained by taking readings with both springs on certain sirups of intermediate consistency and calculating as follows:

$$\text{Spring factor} = \frac{\text{reading with light spring}}{\text{reading with heavy spring}} = 7.0$$

Subsequent correlation experiments with high density sirups proved this factor to be valid.

To obtain a consistency reading, a 350-ml. (12-ounce) open end can (outside diameter 6.7 cm., $2^{11}/_{16}$ inches; height 12 cm., $4^{13}/_{16}$ inches), is filled with the sirup sample and then centered and clamped on the turntable by means of setscrews. The can is locked in position and the table raised until the level of the sirup coincides with the mark on the paddle shaft. The motor switch is then turned on and the torque created causes the pen arm to

describe an arc on the recorder paper. Inasmuch as malt sirups show no thixotropic or rheopectic properties, the pen will draw a vertical line on the graph as the recorder mechanism moves the paper. The paper furnished with the instrument is graduated in "consistency units" from 0 to 1000, so that the position of the recorded line may be numerically evaluated.

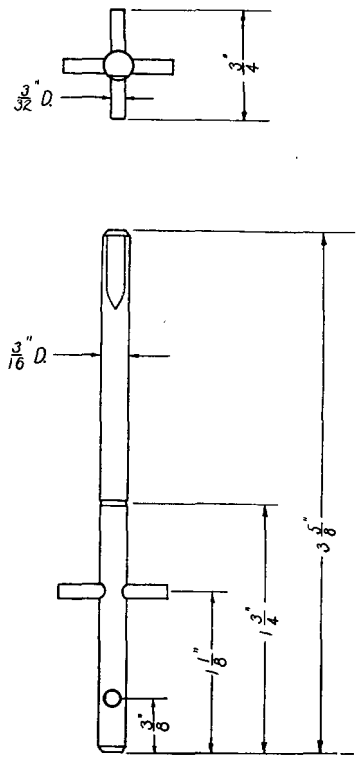

Figure 1. Viscometer Paddle

The three variables, density, consistency, and temperature, are expressed in the following units: density in degrees Baumé (modulus 145), consistency in Brabender "consistency units," and temperature in degrees Centigrade.

In order to determine the relationship between density and consistency, a series of sirup samples of varying densities was required. To obtain these, samples of a malt sirup were taken from a plant evaporator at various stages during the "finishing off" process, giving a density range of 39.5° to 42.5° Bé. Densities were determined by the A.O.A.C. pycnometric procedure (diluting 50.0 grams of sirup with an equal weight of water), and consistency readings were taken at a constant temperature of 20° C.

Similarly, to determine the relationship between consistency and temperature, samples of the finished sirup (constant density) were tempered to 20°, 29.5°, 35°, and 40.5° C. and consistency determinations were made at those temperatures.

MATHEMATICAL ANALYSIS OF DATA

The coordinate points relating density to consistency appeared to express an exponential function of the general type, $Y = b10^{MX}$. When such an exponential curve is plotted on semilogarithmic paper, the equation becomes a straight line expressed as log $Y = MX + \log b$. Figure 2 shows the relationship between the logarithms of the consistencies and the respective densities at 20° C.

In order to evaluate the constants M and b, the regression line was calculated from the experimental values and found to be

$$\log C_{20°} = 0.413\ (°\text{ Bé.} - 39) + \log 20$$

where $Y = C_{20°}$ = consistency units at 20° C.
$M = 0.413$
X = ° Bé.
b = 20 (intercept on the Y axis)

(The constant, 39, compensates for shifting the intersection of the X and Y axes from 0° to 39° Bé.) By transposition, the above equation may be expressed:

$$\text{Degrees Baumé} = \frac{\log C_{20°} - \log 20}{0.413} + 39 \qquad (1)$$

which permits the calculation of sirup density in degrees Baumé from the Brabender consistency reading made at 20° C.

When the temperature-consistency data were graphed on logarithmic paper, a straight line was obtained, which indicated that the relationship was that of a power function whose general equation is $Y = bX^M$ or the straight-line form, log $Y = M \log X + \log b$. The regression line was calculated and the consistency-temperature equation for this particular sirup was found to be:

$$\log C_t = -2.63 \log t + 5.95824 \qquad (2)$$

shown in Figure 3.

An expression combining the three variables, degrees Baumé, consistency, and temperature may now be set up. From Equation 2 the relationship between consistency and temperature at 20° C. is

$$\log C_{20°} = -2.63 \log 20° + 5.95824 \qquad (3)$$

Subtracting Equation 2 from Equation 3:

$$\log C_{20°} - \log C_t = -2.63 \log 20° + 5.95824 - 5.95824 + 2.63 \log t$$

$$\log C_{20°} = -2.63 \log 20° + 5.95824 - 5.95824 + 2.63 \log + \log C_t$$

$$\log C_{20°} = 2.63\ (\log t - \log 20°) + \log C_t \qquad (4)$$

By substituting this expression for log $C_{20°}$ in Equation 1, the desired relation among the three variables is obtained.

$$°\text{Bé.} = \frac{\log C_t + 2.63\ (\log t - \log 20°) - \log 20}{0.413} + 39 \qquad (5)$$

By means of Equation 5 the density of sirups of this particular type may be calculated from the Brabender consistency value at any temperature. To eliminate the calculation of densities from consistency readings, a chart may be constructed as shown in Figure 4. Each diagonal line represents the relationship between temperature and consistency for a specified Baumé value. For this particular sirup these "iso-Baumé" lines are calculated over a range between 41.5° and 43.0° Bé. in steps of 0.1° Bé. The density is read from the chart by determining the value of the diagonal line nearest the point of intersection of the coordinate values of temperature and consistency.

DISCUSSION

When the consistency-density-temperature relationships were investigated for other types of malt sirup, it was discovered that the composition of the sirup affected these relationships (*4*). Comparable composition effects have been clearly shown for corn sirups by Miller and Mench (*5*). Consequently, separate curves must be developed for each type of sirup analyzed. In the authors' laboratories, this has been done for five different types of sirup. Table I shows average approximate analyses.

These typical commercial sirups were produced from mashes varying in composition from all barley malt and no adjunct (Types IV and V) to those containing 40% barley malt and 60% adjunct (Type I). Intermediate sirups were made from mashes

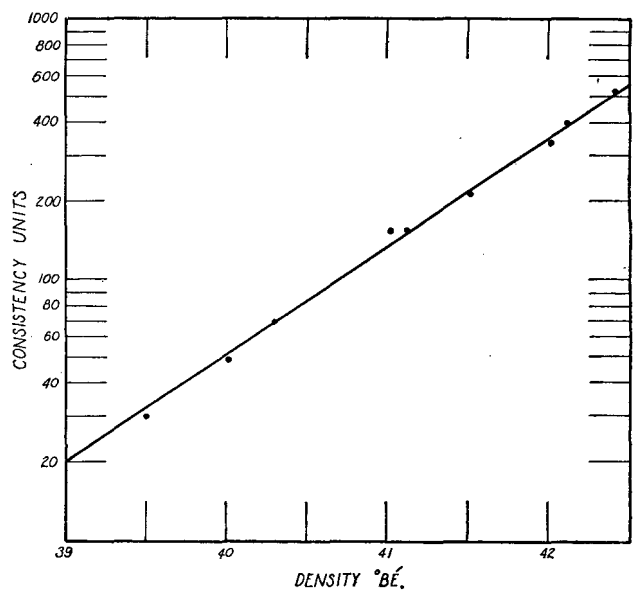

Figure 2. Density-Consistency Relationship

Table I. Analyses of Malt Sirups

Sirup Type	Density, ° *Bé.*	Total Solids, %	Protein, %	Ash, %	Reducing Sugars as Crude Maltose, %	Undetermined (Dextrins, Gums, etc.), %
I	42.1	79.3	2.3	0.8	67.2	9.0
II	42.1	79.3	3.5	1.1	58.0	16.6
III	42.6	80.3	4.3	1.2	72.1	3.2
IV	42.5	80.1	6.1	1.5	70.8	2.1
V	43.0	81.1	4.7	1.3	59.6	15.5

Table II. Comparison of Density Values Obtained by Consistency and Specific Gravity Methods

(Density, ° Baumé)

Type	Specific Gravity Method	Consistency Method	Difference
I	41.5	41.5	0.0
	42.6	42.7	+0.1
	42.2	42.2	0.0
	42.2	42.1	−0.1
II	42.0	42.0	0.0
	42.3	42.2	−0.1
	42.2	42.1	−0.1
	42.0	42.0	0.0
III	42.8	42.7	−0.1
	42.2	42.3	+0.1
	43.0	43.0	0.0
	42.4	42.3	−0.1
IV	42.4	42.5	+0.1
	42.6	42.6	0.0
	43.1	42.9	−0.2
	42.3	42.3	0.0
V	43.2	43.4	+0.2
	42.5	42.6	+0.1
	42.6	42.5	−0.1
	43.4	43.4	0.0

consisting of 75% barley malt and 25% adjunct (Types II and III). The brewing procedures used varied from low temperature diastatic processes (Types III and IV) to high temperature nondiastatic processes (Types II and V).

Although the sirup composition shows great variation among the different types, there are only insignificant changes between batches of the same type, owing to rigid standardization of the mash bills and brewing procedures. Thus, when the temperature-consistency and density-consistency relationships have been determined for any particular type of sirup, those relationships can then be applied to subsequent batches of the same type of sirup. Daily use of this consistency method for approximately two years has definitely established its reliability and accuracy.

To illustrate the degree of correlation between density values obtained by the specific gravity method and those calculated from consistency readings, twenty pairs of Baumé determinations

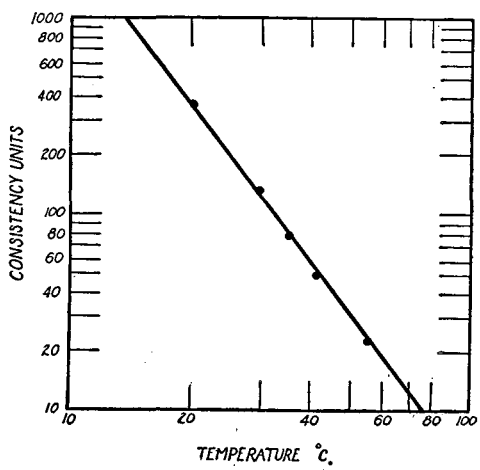

Figure 3. Temperature-Consistency Relationship

are listed in Table II. These values cover the normal range of densities of the five types of sirup mentioned above.

The advantages of the consistency method of determining density are: no dilution of sirup required, no weighing involved, no tempering to standard temperature, no operative skill required, great time saving over other methods, and record of reading obtained for future reference.

The use of the consistency method has greatly shortened the time previously used for density determinations. It has been estimated that the time required for one operator to determine the densities of six sirups by the specific gravity procedure is between 2.5 and 3.0 hours. The same number of determinations require approximately 30 minutes when the Brabender viscometer is used. Density-consistency and temperature-consistency equations have also been developed for corn sirup, molasses, and invert sugar sirup. Because the fundamental equations are of the same form as those developed for malt sirups, the consistency method of density determination should be applicable to such sirups.

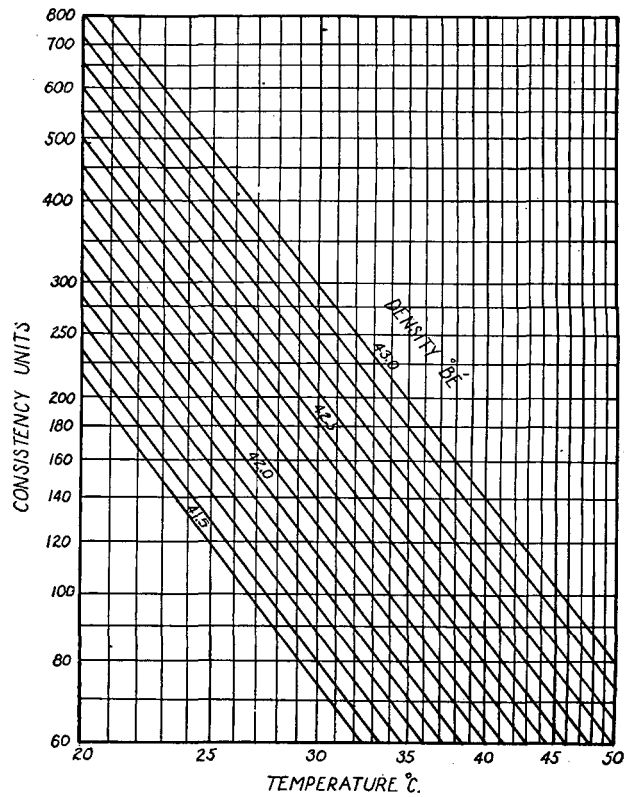

Figure 4. Temperature-Consistency-Density Relationship

ACKNOWLEDGMENT

The authors acknowledge their appreciation of the assistance of Beatrice Hjelte in determining the pycnometric densities of all sirup samples used in this investigation.

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Determination of Water Vapor

Thermal Conductivity Methods

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A saturator capable of delivering a stream of gas containing saturated water vapor in the temperature range 0.5° to 45° C. and at rates up to 1000 ml. per minute is described. This saturator is capable of delivering gas having a water vapor concentration constant to ±0.01% by volume. The limitations, advantages, and unique characteristics of the thermal conductivity method of gas analysis are discussed with particular reference to the quantitative determination of water vapor in gas mixtures. Recording equipment of this type can be made to have excellent constancy of calibration and zero stability. The useful dew point range for water vapor in air, nitrogen, or oxygen is −18° C. (0° F.) to 50° C. (122° F.) and from 80° C. (176° F.) up. These dew points correspond, respectively, to approximately 0.16, 12.3, and 47.4% water vapor by volume. A method for field calibration of equipment of this type without the use of gas-water vapor mixtures is described in the present paper.

THE water vapor content of gas mixtures, particularly air, is a subject that has prompted a variety of investigations over the years. Several well-known methods for the quantitative determination of water vapor have been discussed fully in the literature. On the other hand, the application of the thermal conductivity method to this problem offers some unique possibilities that are not generally known or appreciated. One purpose of this paper is to indicate the advantages, limitations, and scope of the thermal conductivity method as applied to the quantitative determination of water vapor. Another is to discuss steps taken in this laboratory to devise adequate apparatus and methods for the calibration of humidity measuring apparatus.

THERMAL CONDUCTIVITY METHOD

Because the rate at which heat is conducted is not the same for all gases, it is possible under appropriate conditions to determine the composition of a gas mixture by measuring its thermal conductivity, or by comparing its thermal conductivity with that of a reference gas. Since 1904 this method has been under investigation and has been applied successfully in many industrial gas analysis problems. In 1924 Palmer and Weaver (*6*) reported the results of a somewhat extended investigation made at the Bureau of Standards, but their investigation did not include analysis for water vapor.

THERMAL CONDUCTIVITY MAXIMA OF WATER VAPOR–GAS MIXTURES

Weber (*10*) pointed out the possibility of thermal conductivity maxima occurring in binary gas mixtures when the thermal conductivities of the two components are nearly equal. He was unable to predict such a mixture by calculations based upon known molecular constants. Daynes (*1*, *2*) later found experimentally that ammonia-air and water vapor–air mixtures exhibit such maxima. Grüss and Schmick (*3*) then extended the theoretical work of Weber by refining the kinetic theory of binary mixtures and predicted a number of mixtures that should exhibit thermal conductivity maxima. Subsequent experimental work confirmed their predictions.

The occurrence of a thermal conductivity maximum for water vapor–air mixtures has perhaps discouraged application of this method, as indicated by Leone (*5*). This is unfortunate, as the occurrence of the maximum only precludes operation in the dew-point range of 50° to 80° C. (12 to 47% water vapor by volume) in air. On the other hand, there are many problems involving the determination of water vapor in air below 50° and above 80° C. dew point. There are, furthermore, numerous other gas–water vapor systems for which no such maxima occur. For many of these applications the thermal conductivity method is satisfactory and, in some cases, even to be preferred above all other methods.

The existence of the thermal conductivity maximum for mixtures of water vapor and air was confirmed by the author in 1934, and more recent observations in this laboratory indicate similar maxima for water vapor in nitrogen and oxygen. On the basis of the theory it might be expected that a maximum would also occur for water vapor in carbon monoxide and possibly in acetylene, ethylene, and ethane, although no experimental confirmation has been reported. The existence of thermal conductivity maxima are not to be expected for mixtures of water vapor and gases other than those cited above.

INSTRUMENTATION FOR THE THERMAL CONDUCTIVITY METHOD

As early as 1921 Shakespear (*8*) reported the successful use of the hot-wire method (katharometer) under laboratory conditions for the measurement of the humidity of air down to a temperature of −11° C. Rosecrans (*7*) later described a more sensitive thermal conductivity type of apparatus for use in conjunction with an industrial recording instrument for continuously recording relative humidity at constant temperature. Since that time appreciable improvements in the design of thermal conductivity cells have been made with particular emphasis on freedom from corrosion and consequent calibration shift. For a decade or more, thermal conducitivity cells of robust construction have been available in which substantially all areas exposed to the gas sample are of glass or ceramic material. Such cells have suitable sensitivity, stability, robust construction, and corrosion resistance to justify application of this method for quantitative determination of water vapor in gas mixtures. The author and co-workers have made use of such conventional thermal conductivity gas analysis components to develop a complete recording apparatus for humidity measurements. Equipment of this general form is suitable for laboratory or plant use and has already been applied to the recording of dew point in connection with meteorological observations. Briefly, the apparatus consists of a thermal conductivity cell assembly and sampling unit capable of installation out of doors, and an industrial type of recorder and voltage regulator. The recorder and voltage regulator must be afforded protection equivalent to indoor installation.

This equipment has good zero stability and will maintain its calibration for months, provided the gas sample is clean. A suitable filter is provided for removing gross suspended contaminants. The recorder reading for dry air seldom shifts by more than the equivalent of ±0.01% water vapor by volume between weekly zero checks. The over-all time of response

(including the filter) at the normal sampling rate of 200 ml. per minute is from 3 to 4 minutes for 90% of a given change, with an initial response of about 0.8 minute.

In all of his work the author considered the gas to be dry after passing it through indicating Drierite (calcium sulfate) and then through anhydrous magnesium perchlorate. Gas thus treated should have a dew point lower than −70° C., being equivalent to less than 0.001% water vapor by volume. This concentration of water vapor is about equal to the sensitivity of the apparatus used and is much lower than the zero stability of the apparatus demands.

METHODS OF CALIBRATION

The thermal conductivity method is a secondary one requiring calibration against some primary standard. Walker and Ernst (*9*) have described a rather elaborate apparatus for mixing dry air and nearly saturated air in definite proportions to obtain air samples of different humidities. Such a system depends upon precise maintenance of flow ratios for a given setting and must be calibrated by some means such as removing and weighing the water in a known volume of mixture. It has the advantage of being capable of delivering a continuous stream of conditioned gas and does not require recirculation. This is a great convenience in calibrating apparatus of the thermal conductivity type.

The author has investigated several methods of accomplishing the same purpose, and an apparatus has been developed which has been in use for almost three years, giving remarkably consistent results. It appeared desirable to avoid the use of mixing devices and to rely upon a saturator capable of delivering water vapor saturated at controlled temperatures above the freezing point.

Earlier experiences in this laboratory with saturated salt solutions and sulfuric acid solutions indicated inconsistent results except over short periods of time. Values obtained by passing gas over large surfaces of saturated solutions of sodium chloride or ammonium chloride and potassium nitrate did not agree with those for a water saturator at equivalent temperatures. This may have been due to less reliable vapor pressure data for saturated salt solutions or failure to measure the temperature of the actual portion of the system in equilibrium at a given time. Furthermore, the data obtained with saturated salt solutions were erratic and not consistent from day to day, indicating that equilibrium was never more than approximately attained. There were definite indications of fluctuation of the system between conditions representing approximate saturation and supersaturation of the salt solutions used. Stirring failed to remedy this situation.

The type of water saturator finally selected as most satisfactory for temperatures above freezing is shown in Figure 1.

This unit is constructed of brass and copper tubing to provide good heat transfer for rapid temperature equalization. The entire saturator exclusive of water reservoir is submerged in a temperature-controlled oil bath, and the equilibrium temperature is determined by means of a platinum resistance thermometer inserted into the upper chamber of the saturator. This chamber is designed to remove entrained moisture and to allow time for equilibrium to be established. A Mueller bridge is used to determine the resistance of the thermometer.

The saturator has been tested for flow rates up to 1000 ml. per minute and found to deliver gas of substantially constant humidity for any flow in that range, provided the oil-bath temperature is adjusted to give equal resistance thermometer readings at each flow. It has been observed that the resistance thermometer in the saturator indicates a temperature slightly lower than the mean temperature of the oil bath and that this difference increases as the flow rate increases. This is undoubtedly due to the greater rate of evaporation at higher flows. For instance, when dry air is the influent gas, the difference between saturator temperature and mean oil-bath temperature is neg-

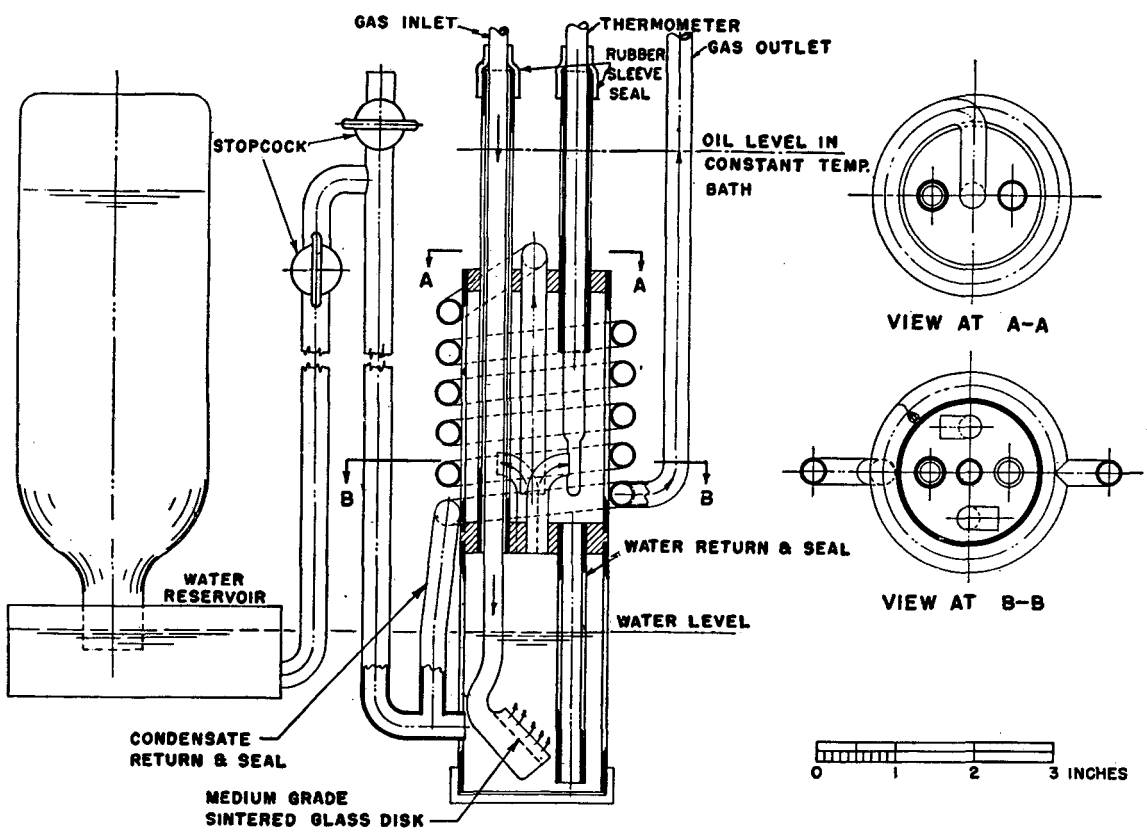

Figure 1. Water Saturator

ligible at very low flow rates, but amounts to 0.05° C. for a flow of 200 ml. per minute at a mean oil-bath temperature of 26.3° C.

The unusually good reproducibility (±0.01% water vapor by volume) at any temperature from 0.5° to 45° C. and freedom from the effects of rate of gas flow up to 1000 ml. per minute are indicative of operation that closely approximates the attainment of equilibrium. The small difference in temperature between the equilibrium chamber and the mean oil-bath temperature is further supporting evidence of reliable operation. The response of the saturator to temperature changes is rapid, as indicated by fluctuations of the effluent humidity in phase with and equivalent to the magnitude of the temperature control cycle of the oil bath.

In order to evaluate the efficiency of operation of the saturator, its output has been analyzed by comparison with an independent method which comprised the absorption and weighing of the water vapor in a known volume of effluent gas from the saturator.

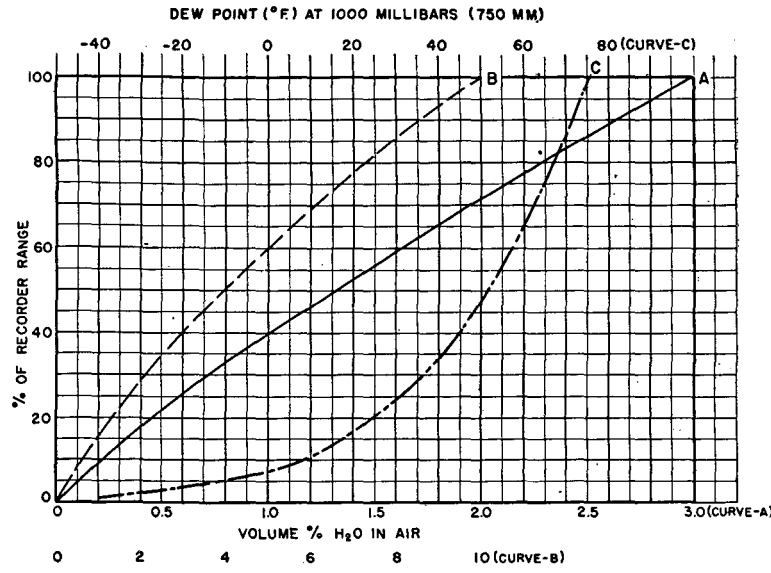

Figure 2. Calibration Curves

Comparisons were made at two temperatures of operation—i.e., approximately 10° and 22.5° C.—and in each experiment the measured volume of effluent (on a dry gas basis) was of the order of 7.5 liters, which permitted a weighing precision of better than 0.2%. In these experiments the rate of gas flow through the saturator was varied from one experiment to another within the limits of 160 to 300 ml. per minute. At the conclusion of each experiment the mean concentration of water vapor (volume per cent) in the effluent was first computed from the mean temperature of the saturator, using accepted data for the vapor pressure of water, and then from the weight of water absorbed from the known volume of effluent gas. A total of 7 runs was made at 22.5° C. and 5 at 10° C.

Table I. Gravimetric Check of Saturator Efficiency

Mean Temp. of Saturator, ° C.	Rate of Gas Discharge, Ml./Min.	Volume Per Cent Water Vapor in Effluent Gas: From vapor pressure, X_p	From weight, X_g	Difference, $X_p - X_g$
22.701	300	2.719	2.695	0.024
22.724	300	2.730	2.731	−0.001
22.531	250	2.686	2.699	−0.013
22.671	220	2.717	2.695	0.022
22.693	220	2.721	2.702	0.019
22.695	220	2.728	2.721	0.007
22.635	200	2.713	2.733	−0.020
9.896	280	1.196	1.196	0
9.907	265	1.214	1.193	0.021
9.922	210	1.217	1.187	0.030
10.023	170	1.220	1.219	0.001
10.060	160	1.207	1.218	−0.011

A summary of the pertinent data is given in Table I. From these limited data it appears that the two methods agree within the observed reproducibility of saturator operation. The mean difference of all observations at 10° C. is 0.0082% by volume, which is equivalent of 0.10° C. (0.18° F.) in terms of the equilibrium temperature. At 22.5° C. the mean difference is 0.0054% by volume or equivalent to 0.033° C. (0.59° F.). In both series of experiments the computation based upon the saturator temperature yielded slightly higher mean values for water vapor concentration.

A limited amount of experimental work has been done at subfreezing temperatures using a modification of the apparatus described above. The modification consists of a condensing chamber maintained at subfreezing temperature by use of "freezing mixtures." The presaturator is operated at a temperature slightly above freezing and its effluent is then passed into the subfreezing condensing chamber. By this means the author has endeavored to study the uncertainty of water vapor equilibria at subfreezing temperatures. According to Keyes and Smith (4), "the water content of air in equilibrium with ice does not correspond to what would be expected on the basis of Dalton's law from the known vapor pressures of ice, but rather to the pressure of subcooled water. The author's observations appear to indicate that this statement is true when a "condensing equilibrium" condition prevails—that is, the vapor pressure of the influent is high enough to require continuous condensation on the ice surface. On the other hand, if an "evaporation equilibrium" prevails on the ice surface, the vapor pressure of the effluent air appears to correspond to that of ice. Studies have not been sufficiently critical to state this as an indisputable fact, but the available evidence appears to justify such a statement.

Typical calibration curves for a thermal conducitivity type of recording apparatus as determined by the above methods are shown in Figure 2, in which curves A and B are in terms of volume per cent water vapor. Curve C represents the dew point calibration at standard 1000 millibars (750 mm.) pressure for a full scale recorder range equivalent to 3% water vapor by volume.

The foregoing discussion of calibration methods indicates clearly that direct humidity calibration is an operation not easily done outside a well equipped laboratory. Considerable thought has been given to field checking methods. A device for providing saturated gas at the ice point has been developed and is simple enough for portable use, but saturated water vapor at temperatures above or below the ice point is more difficult to provide outside the laboratory. However, with the thermal conductivity method an indirect calibration procedure may be employed. It is only necessary to provide a gas mixture having a thermal conductivity equal to that of a given water vapor–gas mixture at the operating temperature of the cells used. For example, water vapor–air mixtures can readily be simulated by oxygen-air mixtures and for the operating conditions of his cells the author has used the following mixtures:

Approximate Mixture, %	Actual O_2 in Mixture (Orsat Analysis), %	Water Vapor Equivalent (Vol. %)
7.9 O_2 + 92.1 air	27.3	0.785
14.8 O_2 + 85.2 air	32.7	1.61
17.4 O_2 + 82.6 air	34.7	1.92

Other mixtures can be prepared to simulate various concentrations of water vapor in any gas. A satisfactory procedure for making such mixtures is:

Compute the approximate composition required to have the desired thermal conductivity.

Prepare the mixture by the usual method of partial pressures and allow sufficient time for thorough mixing. (Storing cylinders in a horizontal position for at least 48 hours is usually sufficient to obtain thorough mixing.)

Pass the gas mixture through a previously calibrated thermal conductivity cell to determine the equivalent water vapor concentration of the mixture.

CALIBRATION UNITS

Inasmuch as a thermal conductivity cell is responsive to the ratio of the partial pressure of the variable constituent to the total pressure, it is common practice to express performance in terms of volume per cent. A given equipment properly calibrated in volume per cent of water vapor in any gas needs no secondary corrections for variations in sample temperature and pressure. However, it is necessary in transferring the humid sample from the sampling point to the measuring unit to avoid temperature or pressure changes that would cause condensation of moisture from the sample. No special arrangements need be made if ambient temperature is always above the dew point of the sample.

Calibration units other than volume per cent may be employed, but in some cases appropriate corrections for temperature or pressure must be made. A few typical cases are listed in Table II, and Figure 3 shows the necessary pressure corrections to be applied when a dew point calibration based upon 1000 millibars (750 mm.) is used.

FEATURES OF THERMAL CONDUCTIVITY METHOD

The general limitations of the thermal conductivity method are: It is a secondary method requiring calibration. The method is not specific to a given gas, as response is due to the total change thermal conductivity of the gas mixture (see Table III).

Table II. Calibration Units and Corrections

Calibration Unit Used	Correction Required
Ratio of vapor pressure to total pressure, or mole fraction	None
Specific humidity or mixing ratio (weight of water vapor per unit weight of dry gas)	None
Dew point at standard pressure	Pressure
Absolute humidity at standard temperature and pressure (weight of water vapor in unit volume of mixture)	Temperature and pressure
Relative humidity at standard temperature and pressure	Temperature and pressure

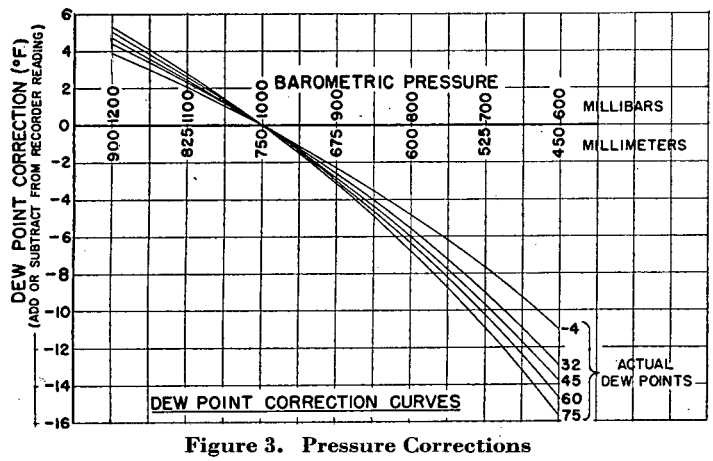

Figure 3. Pressure Corrections

Table III. Effect of Contaminants on Accuracy of Observed Concentration of Water Vapor in Air When No Compensation is Applied

	Contaminant	Concentration Change Necessary to Cause Error of 0.1% by Volume in Observed Concentration of Water Vapor in Air[a], % by Volume
1	Oxygen	+1.0
2	Ammonia	+0.8
3	Ethane	+0.4
4	Methane	+0.04
5	Hydrogen	+0.005
6	Sulfur dioxide	−0.09
7	Carbon dioxide	−0.2
8	Ethyl alcohol	−0.2
9	Argon	−0.2
10	Methyl alcohol	−0.2
11	Acetylene	−0.3
12	Carbon monoxide	−0.8
13	Ethylene	−4.8

[a] Sign indicates direction of concentration change necessary to cause an increase in observed % H_2O.

The first limitation is not serious, as it represents only preliminary inconvenience in applying the method to a given problem. The second limitation is more fundamental and imposes the necessity for a careful survey and analysis of a proposed application to determine its feasibility. Table III indicates the relative effects of various contaminants on the accuracy of the observed concentration of water vapor in air when no compensation is applied. By use of a differential compensating arrangement it is possible to reduce these effects considerably—for example, the tolerance to traces of hydrogen can be increased a thousand times. Fortunately, there are many applications where only a single component varies, or where two or more components vary according to a predetermined function. Furthermore, the thermal conductivity method is susceptible to a variety of modifications that can reduce an apparently complex application to the equivalent of the simplest case.

In addition to the general limitations already cited the specific limitations of this method as applied to humidity measurements are:

For gas mixtures containing predominantly air, nitrogen, or oxygen, the method is not applicable in the approximate dew-point range of 50° to 80° C. (12 to 47% water vapor by volume). Other gas mixtures for which a similar limitation may be necessary have been indicated.

The stability attainable with currently available equipment limits application of the method to dew points higher than about −18° C. (0° F). Quantitative determination of the water vapor content of gas saturated at −18° C. can be made to ±5% and the precision of the method increases rapidly with increasing dew point.

The nature of the limitations cited is such that there would appear to be many applications for which the thermal conductivity method can be used to advantage, particularly when recording or process control are desired.

Attention is called to the following advantages of the method as applied to humidity measurements:

It is easily adapted to recording and/or control.

It is possible to calibrate apparatus to be direct reading for a variety of units, and in some cases to provide automatic compensation for uncontrolled variables.

It is possible to measure: difference in humidity from a standard condition, and ratio of an existing humidity to a standard condition.

Ordinarily no water vapor need be added to nor removed from the system. This is of particular importance for small closed systems.

The temperature of the sample has no detrimental effect upon the measurement, provided it remains above the dew point of the sample and below the temperature of the cell wall.

Long-time stability of calibration can be achieved.

Calibration check can be made by using appropriate gas mixtures stored under pressure.

No cooling system is required as an integral part of the measurement, in contrast to the condensation method of determining dew point.

Maintenance of wick or spray devices is eliminated.

The extensive industrial and laboratory use of the thermal conductivity method of gas analysis for many years is generally indicative of its broad possibilities. However, relatively few applications have been reported for the determination of water vapor concentration and the material presented in this paper is intended to aid in the evaluation of this method in terms of any specific problem of humidity measurement.

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Gas Thermometer for Automatic Control of Low Temperatures

Application to Separation of Gases by Isothermal Distillation

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A gas thermometer is described for the automatic control of temperatures down to −180° C. as required in the separation of gases by isothermal distillation. The helium-filled reservoir of the thermometer is located in a copper block which surrounds the distilling tube. A constant heat loss from the copper block and the distilling tube to liquid air is offset by intermittent heating which is controlled through a relay by the position of the mercury meniscus in the manometer. The thermometer can be preset to control the temperature at any desired point by proper adjustment of the volume of mercury in the manometer. The temperature is controlled to approximately ±0.3° C. The apparatus is simple to construct and is easy to operate.

THE separation of gases at low temperatures and pressures by isothermal distillation as described by Shepherd (*4*), Ward (*6*), and Savelli, Seyfried, and Filbert (*2*) requires the use of distillation temperatures in the range from −75° C. down to the boiling point of liquid air. Usually the distilling tube is placed inside a copper block which has a small electrical heater wound on the outside. The copper block is placed in a test tube immersed in liquid air to provide a small but constant heat loss. The temperature is maintained constant by manually adjusting the current through the heater so as just to offset the heat loss to the liquid air. The method described by Ward (*6*) and Savelli, Seyfried, and Filbert (*2*) uses a series of four distilling tubes maintained at progressively lower temperatures. The temperature of each distilling tube is increased after each fraction has been removed. In applying this method, the initial adjustment and the maintenance of the temperatures at the prescribed values with manual controls were found to be rather difficult. The gas thermometer described in this paper provides a simple means for both the adjustment and automatic control of these temperatures.

APPARATUS

A schematic diagram of the temperature control unit developed for this purpose is shown in Figure 1. Briefly, the operation of this control unit is as follows:

A gas reservoir which is filled with helium and is connected through a small tube to a manometer is maintained in close thermal contact with the distilling tube. Changes in temperature of helium in the reservoir cause corresponding changes in the position of the mercury in the manometer. These changes actuate a relay through suitable electrical contact points sealed into the manometer. The relay cuts off and turns on the current through a small electrical heater which is also in thermal contact with the distilling tube. The temperature control is effected by balancing this intermittent heat input against a constant heat loss to liquid air. The temperature maintained by the unit can be adjusted by varying the volume of mercury in the manometer.

The close thermal contact required between the distilling tube, the helium reservoir, and the electrical heater is secured by surrounding the distilling tube with a heavy copper block; the heater is wound around the outside of the block and a cavity in the walls of the block serves as the helium reservoir. The distilling tube fits into a hole $^5/_8$ inch (1.6 cm.) in diameter, drilled down the center of the block. A little carbon tetrachloride is added around the distilling tube to improve the thermal contact. The nine vertical holes $^5/_{16}$ inch in diameter drilled into the walls of the block serve as the helium reservoir. The holes are all connected by a channel cut around the top of the block; the gas space is sealed off by soldering a ring into the top of this channel. The volume of the resulting reservoir is approximately 53 ml. The gas connection to the reservoir is made through a length of hypodermic tubing 0.6 mm. in inside diameter soldered into a copper plug which is in turn soldered into the top of the block. This plug is used to provide an opening sufficiently large to permit filling the reservoir with water for the purpose of determining the volume before final assembly.

The manometer as shown in Figure 1 is constructed of glass tubing 6 mm. in inside diameter and has two tungsten contacts sealed into the right leg. The lower contact is located near the bottom of the manometer, where it is always immersed in the mercury. The upper contact is located approximately 30 cm. above the bottom of the manometer. A check valve is located

just above this contact to prevent mercury from accidentally entering the copper block. The gas volume between the upper contact point and the hypodermic tubing to the reservoir is kept as small as possible. In the four thermometers constructed this space varied from 0.5 to 1.0 ml.

The left leg of the manometer has an over-all length of approximately 110 cm. The top of this leg is connected to a vacuum pump or alternatively to a supply of helium. A 120° type, 3-way stopcock is placed in the left leg 2 to 5 cm. above the bottom and is connected through a needle valve to a steel mercury reservoir. By means of vacuum or by air pressure above the mercury in the reservoir, it is possible to change independently the position of the mercury meniscus in either leg of the manometer. When four distilling tubes are used, the manometers are all connected to the same mercury reservoir.

The two tungsten contacts are connected through a source of 6 volts direct current to a relay which is arranged so as to turn off the current through the heater wound around the copper block when the electrical connection is broken at the upper tungsten contact, and vice versa. A small rheostat is placed in the heater circuit for the purpose of regulating the current. It was found to be essential for proper operation to fill the manometer with a caustic solution and to pass a current between the two tungsten contacts from a 6-volt alternating current source sufficiently long to remove the oxide coating from the tungsten.

The volume of the gas reservoir in the copper block and the volume of the gas space in the manometer must be determined before or during the assembly of the apparatus. The volume of the reservoir in the copper block was determined from the weight of water required to fill this space. The manometer volume was taken as the gas volume between the reservoir in the copper block and the mercury meniscus in the right leg of the manometer when the meniscus is just touching the upper tungsten contact. The volume of the hypodermic tubing and the volume of the glass capillary on the manometer were calculated from their physical dimensions. The volume between the mercury meniscus and the capillary was determined from the diameter of the left leg of the manometer and the lowering of the mercury in this leg required to move the meniscus in the right leg from the tip of the tungsten contact to a position up against the check valve at the bottom of the capillary.

Preparation of Apparatus. After assembly of the apparatus and determination of the two volumes as described above, the thermometer is ready for filling with helium.

All mercury in the manometer is withdrawn first to permit evacuating the helium reservoir through the left leg of the manometer. Mercury below the 3-way stopcock which cannot be drained out to the mercury reservoir is removed by suction through a small tube inserted through the stopcock after removing the plug.

After the entire apparatus has been evacuated, including the reservoir in the copper block and the connection between the 3-way stopcock and the mercury reservoir, the reservoir in the copper block is filled through the manometer with pure helium to a pressure of 600 to 650 mm. The helium is confined in the thermometer by filling the manometer with mercury through the 3-way stopcock. The upper part of the left leg of the manometer is then evacuated.

Temperatures are calculated from a slightly simplified form of the equation used by Southard and Milner (*5*). The simplification consists in assuming that all the connecting tubing between the helium reservoir and the manometer is at the temperature of the gas in the manometer. In view of the very small volume of the gas in the connecting tubing, which is at a temperature intermediate between the temperature of the manometer and the temperature of the reservoir, this simplification does not introduce a significant error in this application. The simplified equation is as follows:

$$T_v = \frac{PV_0}{C}\left[1 + \alpha(T_v - T_0) + \frac{V_m T_v}{V_0 T_m}\right] \qquad (1)$$

where T_v = absolute temperature of helium reservoir, V_0 = volume of reservoir in milliliters as determined at temperature T_0, P = corrected pressure of helium in millimeters of mercury, C = constant determined by calibration at any known temperature, α = cubical coefficient of expansion of copper, V_m = volume of gas space in manometer in milliliters (including volume in hypodermic tubing), and T_m = absolute temperature of V_m.

The value of α, the cubical coefficient of expansion of copper, was taken as 0.000028; this value was calculated from the data of Buffington and Latimer (*1*). Constant C was calculated from the pressure observed when the copper block was cooled to 0° C. In measuring this pressure it is essential that the volume of the mercury in the manometer be adjusted so that the meniscus in the right leg just touches the upper tungsten contact.

Each of the four thermometers constructed was checked at the oxygen point against an oxygen vapor pressure thermometer (*3*). None of the thermometers was in error by more than 0.5° C. When in use the thermometers are checked periodically at the oxygen point as a test for possible leaks.

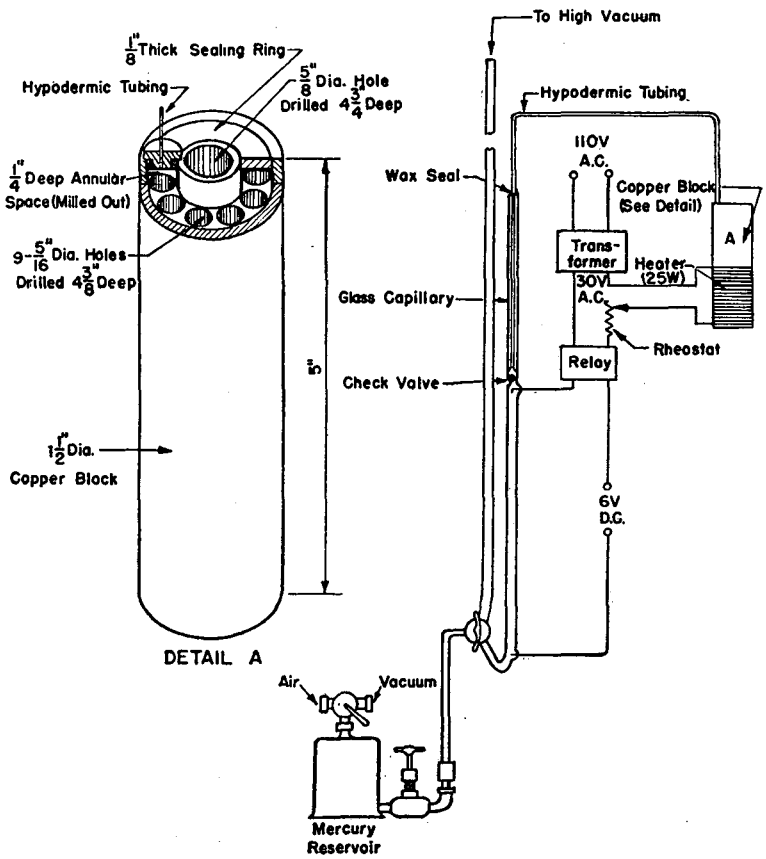

Figure 1. Temperature Control Unit for Isothermal Distillation Apparatus

OPERATION OF THE CONTROL UNIT

The temperature of the distilling tube is adjusted to any desired value by first calculating the corresponding pressure from Equation 1. The position of the mercury meniscus in the right leg is then adjusted just to touch the upper contact point; the meniscus in the left leg is set at a height above the meniscus in the right leg corresponding to the calculated pressure corrected to room temperature. The two legs of the manometer are then connected

together through the 3-way stopcock. If the copper block is at room temperature, the meniscus will fall in the right leg and rise in the left leg.

The copper block is then cooled by direct immersion in liquid air until the meniscus in the right leg is back to a position near the upper tungsten contact. The liquid air is removed, a glass test tube slipped over the copper block, and the test tube immersed in liquid air. The position of the mercury in the manometer then turns the heater current off and on, so as to maintain the meniscus near the contact point. The rheostat in the heater circuit and the depth of the liquid air around the test tube are adjusted so that the off and on periods for the heater are approximately equal in duration. When properly adjusted the temperature remains constant to ±0.3° C.

When it is desired to increase the temperature of the distilling tube to the next value, the height of the mercury in the left leg is reset to the proper position with the mercury in the right leg still at the tungsten contact. Without further attention the temperature quickly rises to the desired value and then is automatically maintained constant at this point.

DISCUSSION

This control unit has been found to be a very satisfactory means of both adjusting and controlling the temperature required for isothermal distillations at low temperatures. The operation of the thermometer is very simple and it requires a minimum of attention from the operator. The apparatus should also be applicable to the control of low temperatures as required in other problems.

For use as both a method of temperature measurement and temperature control the 120° type 3-way stopcock in the manometer should probably be replaced with a T-type stopcock. This type of stopcock has an advantage when measuring temperatures, as it permits adding mercury to both legs of the manometer simultaneously until the meniscus in the right leg just touches the contact point. However, when the main application is the presetting of the control point at some particular temperature and the control of the temperature at this point, the 120° type stopcock is believed to be preferable. Although the location of the stopcock above the bottom of the manometer makes somewhat inconvenient the removal of all the mercury as required when filling the thermometer with helium, it has the advantage of permitting relubrication of this stopcock without loss of the helium from the reservoir.

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Quantitative Colorimetric Microdetermination of Methanol with Chromotropic Acid Reagent

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A rapid, accurate, and specific method for the quantitative determination of methanol is described in which the methanol is oxidized to formaldehyde and the latter measured colorimetrically with chromotropic acid. The method permits the determination of methanol with a relative error of <2%.

FORMALDEHYDE when heated with chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in the presence of sulfuric acid gives rise to an intense violet-red color (*1*, *2*, *4*). The formation of this color is the basis for the analytical method for methanol described below (*7*). This method was developed in connection with a problem requiring a rapid, accurate, and specific determination of methanol for which other available methods, including the Zeisel method for alkoxyl groups, were unsuitable. The specificity of the reaction is indicated by the fact that the following aldehydes do not react with chromotropic acid to give a colored solution: acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, isovaleraldehyde, crotonaldehyde, chloral hydrate, glyoxal, benzaldehyde, and phthalaldehyde. Glyceraldehyde gives a yellow color (*4*).

REAGENTS

Methanol, reagent Merck. Dilute phosphoric acid, 10 ml. of 50% acid diluted to 100 ml. Potassium permanganate, 5% aqueous solution. Sodium bisulfite, reagent Merck, saturated solution. Concentrated sulfuric acid.

Chromotropic acid, Eastman Kodak (P-1613), 2% aqueous solution. Ten milliliters of the acid solution are prepared and stored in a brown bottle. The solution darkens rapidly on standing unless preserved by cold storage.

PROCEDURE

The organic material is weighed into a distilling flask with 4 ml. of water and distilled. Three milliliters of the distillate are collected and diluted according to the expected methanol content (the test solution). One milliliter of the test solution is transferred to a 10-ml. volumetric flask, to which are subsequently added 3 drops of dilute phosphoric acid and 5 drops of potassium permanganate solution (*3*). The solution is kept at room temperature for 10 minutes with occasional swirling to ensure oxidation of the methanol to formaldehyde. Sodium bisulfite is then added dropwise to the solution to reduce the excess permanganate.

The solution is cooled by swirling the flask in an ice bath while 4 ml. of cold concentrated sulfuric acid are added. If a buret is used for the measurement of the sulfuric acid, the stopcock should be lubricated with concentrated sulfuric acid only.

Four drops of chromotropic acid reagent are added to the solution and the 10-ml. volumetric flask is placed in a water bath at 60° C. for 15 minutes, during which time the flask should be swirled occasionally. The flask is removed from the water bath and cooled in an ice bath. Distilled water is added to bring the level to within 2 mm. of the mark on the flask. The flask is stoppered, shaken, and allowed to stand until the solution is at room temperature. Sufficient distilled water is then added to bring the level of the solution to the mark.

A blank is run on 1 ml. of distilled water each day, as chromotropic acid solution darkens with time. The solutions under test and the blank are transferred to the cells of a Beckman spectrophotometer and compared at 5800Å. (*5*). (Evelyn and Coleman photometers have also been employed successfully.) The quantity of methanol in the test solution is then read from an optical density-concentration curve which is determined as follows: A series of aqueous methanol solutions containing from 20 to 100 micrograms of methanol per milliliter of solution is prepared and treated according to the above procedure. The colored solutions are stable for at least 3 days.

The following results are typical of the agreement obtained between the Zeisel and the proposed colorimetric method when methanol was the only alcohol present:

Zeisel, %	Colorimetric, %
5.5	5.0, 5.2
4.8	5.0

The procedure here described may be employed to advantage in the determination of methoxyl groups in methyl esters (*6*).

As an example, a sample of dimethyl tartrate was saponified with 4 ml. of 5 *N* sodium hydroxide. After 3 ml. of distillate had been collected, the latter was diluted and its methanol content determined by the procedure described. The results showed 2.0 moles of methanol as compared with a theoretical value of 2 moles.

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ELECTRON MICROSCOPE GONIOMETRY

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The frequent occurrence of unknown crystals in electron microscopical samples presents the problem of their identification. Their relatively small size severely limits the ordinary methods of identification. The silhouette angles observed on the screen or photographic plate are practically all the data available to the electron microscopist. These angles are functions of the interfacial angles of the crystals and the orientation with respect to the screen or plate. The interfacial angles, constants of a suspected compound as obtained from the literature, can be used to calculate the angles of an orthographic projection of the crystal, which is observed with the electron microscope. A comparison of the angles measured with those calculated may establish the identity of the crystal. It may be possible to determine directly axial elements (axial ratios and interaxial angles) and interfacial angles of unknown crystals. This represents the determination of physical constants with the electron microscope.

THE frequent occurrence of unknown crystals in electron microscopical samples presents the problem of identification. The microscopic size of the crystals or the nature of the sample severely limits the ordinary methods of identification. Precipitated materials, pigments, and by-products, such as calcium carbonate, which is used as the illustration in this paper, are an example of the occurrence of microscopic sizes. Crystals associated with other materials in such a manner that separation is not possible, such as crystals attached to fibers or even to other crystals, especially when common ions are present, illustrate the limits due to the nature of the sample. The history of the samples usually limits the number of possibilities and the problem then can be reduced to confirmation of a suspected identity. The authors were presented with the problem of identification of crystals in electron micrographs with the suggestion that the silhouette angles, practically all the data available to the electron microscopist, be considered as data for the confirmation of identity. It was then realized that crystallographical concepts could be applied to this problem.

ORIGIN AND NATURE OF PROBLEM

Electron micrographs of industrial sludges from the carbon dioxide and sulfuric acid processes for the extraction of cyanamide from crude, commercial calcium cyanamide showed outlines of relatively small crystals (ca. 3 to 5μ in breadth). The nature of the sample suggested that the crystals might be calcite. The confirmation of this hypothesis by a determination of some physical property was desired. The identification of the crystals as the calcite phase of calcium carbonate was confirmed by the use of electron microscope goniometry.

Figure 1 is an electron micrograph of one of the crystals. Its general appearance suggested that the crystal might be one of calcite lying on a rhombohedral face.

Figure 3 is an orthographic projection of a calcite crystal showing only the unit rhombohedron, $r\{10\bar{1}1\}$. The projection plane is parallel to the face $r_1(10\bar{1}1)$ and the crystal is lying on $r_6(\bar{1}01\bar{1})$. The solid lines represent the visible edges, and the dotted lines represent the edges not directly visible.

All faces in this form are identical; however, in the projection, faces parallel to the projection plane show their true size, whereas those at an angle are reduced in size. Angle A is the angle between edges r_2r_6 and r_3r_6, and shows the true value. Angle C is the angle between the projections of edges r_3r_6 and r_3r_4. The plane formed by these edges in space is at an angle to the projection plane: therefore, the angle between the edges as projected is different from the true value. The calculation of this angle, from the axial elements and others such as B and C', is the problem for the electron microscopist. (In this paper interedge angles are considered as internal angles.)

Donnay and O'Brien (*4*) showed how the apparent interedge angles of crystals observed with the optical microscope could be correlated with true interfacial angles and the axial elements. They demonstrated how known methods of crystal drawing and a knowledge of the spherical projection and its derivatives, the stereographic and cyclographic projections, could be applied to graphical calculations.

In electron microscopy, the silhouette angles of crystals are practically the only determinative data available. In this paper are presented the application of microscope goniometry to the study of electron micrographs and the use of silhouette angles for the determination of physical constants by means of the electron microscope. The calculations follow the methods presented by Donnay and O'Brien (*4*).

Calculation of Angles of Orthographic Projection. Figure 4 is a stereographic projection of a calcite crystal showing only the unit rhombohedron $\{10\bar{1}1\}$. It is derived from the interfacial angle $(0001):(10\bar{1}1)$, which is 44° 36′ (Dana, *2*). This projection was constructed with the use of the Wulff net (Donnay and O'Brien *4*).

Point C is the projection of the polar axis of the fundamental sphere of projection and of the c crystallographic axis. The three points, a_1, a_2, and a_3, are the positive poles of the three horizontal axes of the hexagonal system. The three points, r_1, r_2, and r_3 (double circles), are the stereographic projections of the face poles which are above the equatorial plane of the fundamental sphere.

The three points, r_4, r_5, and r_6 (single circles), are the poles below the equatorial plane. The great circles connecting these points are the zone circles, the loci of the poles of all faces parallel to a common direction. The great circle, $FABC$, is the cyclographic projection of face r_1, the face which is parallel to the plane of the orthographic projection in Figure 3.

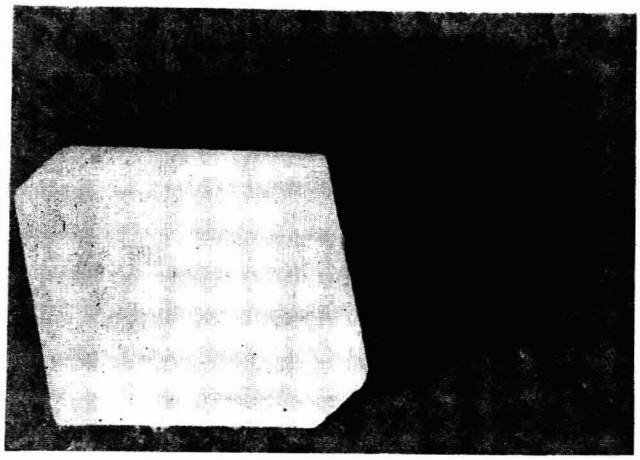

Figure 1. Electron Micrograph of Calcite Shadowed with Antimony at 45° Diagonal 4.5 μ

A cyclographic projection of a crystal face is the stereographic projection of a face circle of a spherical projection. A face circle is a great circle of a spherical projection parallel to a crystal face. A cyclographic projection of a crystal face is the locus of all points 90° from a face pole. In Figure 4, $FABC$ is 90° from r_1.

The graphical solution for the angles of the orthographic projection (Figure 3) is made on the stereographic projection (Figure 4). The intersections of the zone circles, whose projected interzonal angles are desired, with the cyclographic projection of the face parallel to the plane of the orthographic projection describe arcs which are measures of the projected interzonal angles. Their supplements are the angles desired.

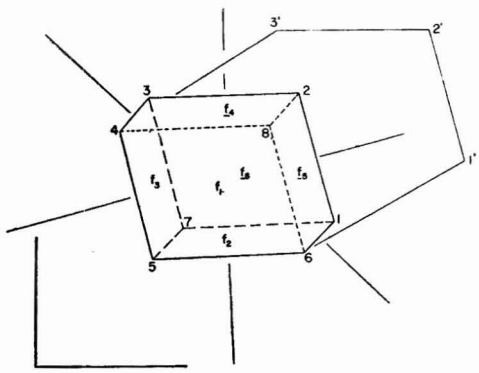

Figure 2. Tracing of Enlargement of Electron Micrograph

In Figure 4, arc AB gives the projected interzonal angle between zone circle LH described by the points L, r_1, r_2, H, r_6, r_4, and zone circle GK described by the points G, r_1, r_3, K, r_6, r_5. It is the supplement of angles A and A' in Figure 3. Arcs BC and CD are the supplements of angles C and C'. Angle B of Figure 3 is interesting in that it is a function of two edges which do not intersect in space, but appear to intersect in the orthographic projection. The edge r_3r_6 is formed in the zone whose projection is circle GK. The edge r_1r_4 is in the zone whose projection is circle LH. The projected interzonal angle is the total arc BC plus CD. Its supplement is angle B. Also, as arc AB is the supplement of $BC + CD$, angle B is equal to arc AB.

Analysis of Electron Micrographs. Figure 1 is an electron micrograph of a calcite crystal shadowed with antimony at an angle of 45° from a distance of 15 cm. The original magnification on the electron microscope was 4900×. The length of the crystal from the vertex at the lower left to that at the upper right was calculated to be 4.5 microns.

The photographic plate was placed in a photographic enlarger and the image was projected onto graph paper. The outlines of the crystal and its shadow were then traced with the use of a straightedge. The optical enlargement was approximately 4×. Figure 2 is the tracing. The two lines forming a right angle in the lower left corner indicate the direction of the lines of the graph paper. The general silhouette suggested that the crystal was rhombohedral and that it was lying parallel to a rhombohedral face. The dotted lines were drawn to show the rhombohedral shape.

The shadow was used to determine the orientation of the crystal with respect to the photographic plate. It was found that points 1′, 2′, and 3′ were equidistant from points 1, 2, and 3, respectively. This was the only possible combination of crystal vertexes with the vertexes of the shadow. This relation also proved that points 1, 2, and 3 were in a plane parallel to the photographic plate. It is seen in Figure 2 that the shadow line from 3′ to 4 passes below point 3 and that the shadow line from 1′ to 6 is visible over its entire length. This shows that the plane of 1, 2, and 3 is above the plane of the substrate. The long-dashed lines 3–7, 1–7, and 5–7 then represent edges on the upper side of the crystal, and the short-dashed lines, 4-8, 6-8, 2-8, edges on the lower side of the crystal. The normals to the edges are also shown. This is the orientation of the crystal of calcite as projected in Figure 3.

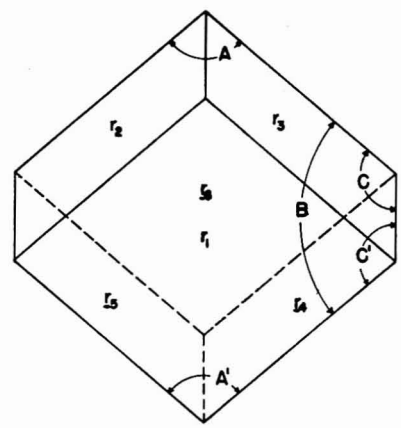

Figure 3. Orthographic Projection of Calcite Crystal Parallel to Unit Rhombohedron

The angles were measured in several ways with the use of a protractor with a least count of 2 minutes. The angles were measured directly; the angles between the perpendiculars of the edges forming the angles gave the supplements of the desired angles. The angles between the perpendiculars and the horizontal graph lines were reduced to interperpendicular angles and then to interedge angles. The sums of the interior angles were checked to see that they were equal to $n - 2(180°) = 720°$. The data for four crystals examined along with the approximate size of the crystals are presented in Table I.

Table I shows that the identity of the crystals as calcite has been confirmed. The error in the measurement of the eight angles of any single crystal varied from 0.9 to 2.7% with an average of 1.6%. The error in the measurement of a definite angle for all crystals varied from 0.0 to 1.5% with an average of 0.8%. The average value of crystallographically identical angles showed an error of less than 0.3%.

The number of independent crystallographic angles that may be measured is a function of the crystal system to which the crystal in question belongs. Complete identification of crystals in the isometric system is not possible. As the three axes of the isometric system are identical, like interfacial angles are identical irrespective of the chemical nature of the crystals. These meth-

Table I. Interedge Angle Measurements

Interedge Angle[a]	Calculated Value: Spher. trig.	Calculated Value: Constr., degrees	Measured Value: I, degrees	Measured Value: II, degrees	Measured Value: III[b], degrees	Measured Value: IV, degrees	Average Value: Four crystals, degrees	Average Value: % error
A	101° 55′	102	100 1/4	100 3/4	102 1/4	103	101 1/2	0.5
A′	101° 55′	102	101	101 1/2	103	103	102	0.0
B	78° 5′	78	79 3/4	79 1/4	77 1/2	77	78 1/2	0.6
	78° 5′	78	79	79 1/4	77 1/2	77	78	0.0
C	129° 2′	129	128 1/2	130 3/4	122 1/2	129 1/4	127 3/4	1.0
	129° 2′	129	132 1/4	128 1/2	135 1/2	127 3/4	131	1.5
C′	129° 2′	129	131 1/2	128 1/2	134 3/4	127 3/4	130 1/2	1.2
	129° 2′	129	130 3/4	130 3/4	121 3/4	129 1/2	127 1/4	1.4
Av. % error for all angles of four crystals								0.8
% error for all angles of a single crystal			1.6	1.1	2.7	0.9	Av. 1.6	
Maximum diagonal, μ			3.3	3.5	4.5	3.3		

	Degrees	% Error
Average obtuse rhomb angle (A,A')	101 3/4	0.2
Average acute rhomb angle (B,B')	78 1/4	0.3
Average side angle (C,C')	129	0.0

[a] See Figure 3.
[b] Crystal of Figure 1.

ods will characterize the crystals as isometric and thus provide a partial identification. For the other crystal systems the number of independent crystallographic angles that may be measured is equal to the number of angles required to calculate the axial elements. The tetragonal and hexagonal systems require only one angle, the orthorhombic two, the monoclinic three, and the triclinic five. The measurement of more angles than these does not in itself increase the certainty of the identification, but does increase the precision of the determination of the characteristic angles, for all angles must be consistent with the symmetry.

DISCUSSION OF ERRORS

A correlative study of Table I and Figures 1 and 3 indicates that the angles that showed the greater errors had the property of being included by a short side. A source of this error is in the adjustment of a straightedge to coincidence with the projected image of a line. The practical limit of resolution of the eye is 0.2 mm. If perfect coincidence is assumed at one end of the line and a deviation of 0.2 mm. at the other, the angle between the straightedge and the line is arc tangent $\frac{0.2}{L}$, where L is the length of the line. This function is illustrated in Figure 5. From this figure, it is seen that an angular deviation of less than 30′(1/2°) requires that the line must be 24 to 5 mm. long; for a deviation of 15′(1/4°) the line must be at least 46 to 47 mm. long. This figure is presented to show the type of error and not its absolute value.

Another source of error is the unevenness of the image of the line. A reduction of the enlargement tends to reduce the irregularities. It is necessary to balance the length factor against the irregularity factor in choosing the enlargement for tracing.

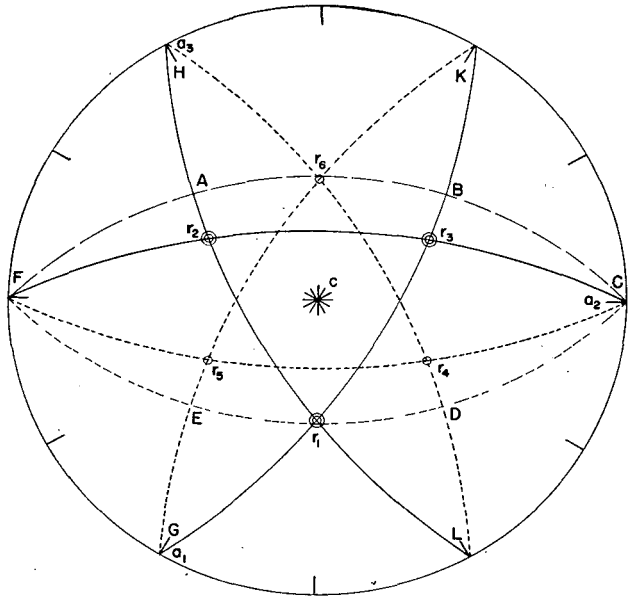

Figure 4. Stereographic Projection of Calcite

CALCULATION OF AXIAL ELEMENTS

From the angles determined on the electron micrograph, the axial elements of the crystal can be graphically calculated. The crystal system can be assumed from a study of the micrographs. The stereographic projection can then be constructed.

The stereographic projection, Figure 6, is derived from the angles measured on Figure 2.

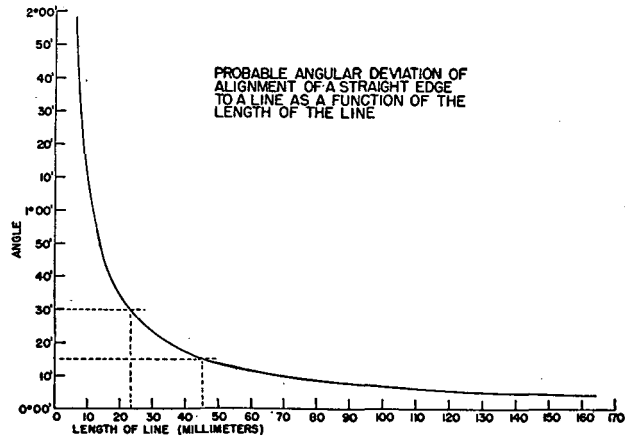

Figure 5. Probable Angular Deviation of Alignment of a Straight Edge to a Line as a Function of Length of Line

Points L_1 and L_2 are the cyclographic projections of the edges f_1f_2 and f_1f_3, respectively. These points, separated by an angle of 101 3/4°, are symmetrically placed with respect to the vertical diameter of the primitive circle. Diameters perpendicular to the radii through L_1 and L_2 are then drawn. These are the zone circles r_1r_2 and r_1r_3. The face poles, r_2 and r_3, lie somewhere along these zone circles. The zone circle, r_2r_3, is found by locating its equivalent pole. If the crystal were lying on any other face, its appearance would still be the same, and the edge f_2f_3 would make an angle of 101 3/4° with either edge f_1f_3 or f_1f_2. The two small circles about L_1 and L_2 whose angular radii are both 101 3/4° intersect at point X, which is the cyclographic projection of the edge f_2f_3. This edge is parallel to the axis of zone f_2f_3 which is perpendicular to the zone circle. The zone circle Fr_2r_3c is 90° from point X. Its intersections with the zone circles r_1r_2 and r_1r_3 locate the face poles r_2 and r_3, which are the face poles of f_2 and f_3.

The next problem is to find the point on the vertical diameter equidistant in angular measure from the three face poles. This point is the projection of the c axis of the crystal. To find c, the circle through $r_1r_2r_3$ must be constructed. Point N, the geometrical center of the circle, is located equidistant from the poles $r_1r_2r_3$. It is found by the intersection of the perpendicular bisectors of the sides of the plane triangle $r_1r_2r_3$. The dotted circle is then drawn. It is now necessary to locate the stereographic center of this circle—that is, the point equidistant in terms of angular measure from all points on the dotted circle. The dotted circle cuts the vertical diameter at Y. Point Y is found to be 89 1/2° from r_1. Point c is then one half of this angular distance from Y and from r_1 or 44 3/4°. Point c is the stereographic center of the circle and is the projection of the a axis. Its distance from r_1 is a measure of the angle between the c axis and the face pole r_1. The formula tan $(c{:}r)$ $\frac{1}{2}\sqrt{3} = c/a$ then gives the axial ratio $a{:}c$. This was calculated to be 1:0.859. The $c{:}r$ angle of calcite measured goniometrically is 44° 36 1/2′ (*2*, p. 512). The axial ratio

from this value is 1:0.854. The error in the angle is 0.3%, and the error in the ratio is 0.6%. Figure 6, when completed, would appear like Figure 4, if the fundamental spherical projection were rotated around the right-left axis until point c was at the north pole—that is, the center of the projection.

All great and small circles in a stereographic projection represent real circles of the spherical projection. However, owing to the tangent function of the stereographic projection, the geometrical center of a circle on the stereographic projection is not the projection of the center of the real circle. The projection of the center of a real circle is the point equidistant from all points on the projected circle in terms of angular, not linear measure.

A compilation of determinative angles for all the crystals reported in the literature is now being prepared in England and its release is expected at an early date. It is based upon the system devised by Barker (*1*) and is well discussed by Donnay (*3*). This should greatly enhance the value of these methods of crystallography in the identification of crystals found in electron microscopical samples.

SUMMARY

The silhouette angles (practically the only analytical data available to the electron microscopist) of crystals observed on electron micrographs can be correlated with interfacial angles. Agreement of calculated with measured angles establishes the identity of the crystals. Furthermore, the silhouette angles may be used to calculate axial elements. These methods represent the determination of physical constants with the electron microscope.

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They also are indebted to their co-workers for suggestions and criticisms.

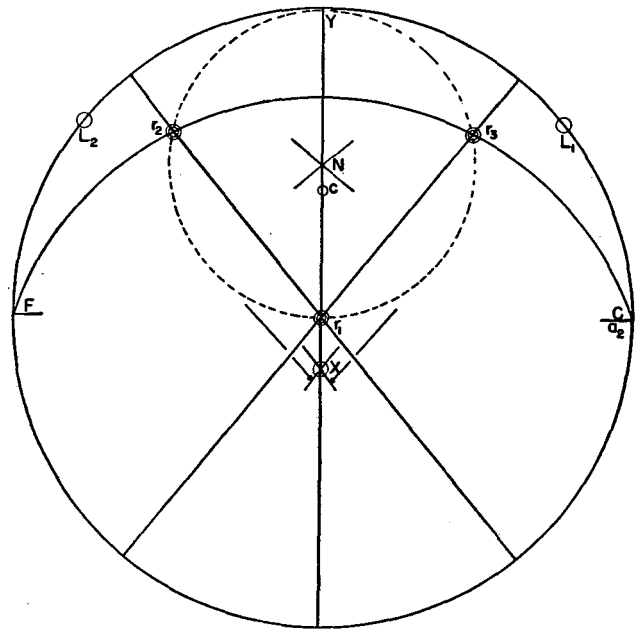

Figure 6. Stereographic Projection of Calcite Using Electron Microscope Goniometric Data

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Volumetric Determination of Microgram Quantities of Acid-Soluble Sulfur

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IN CONJUNCTION with one phase of the research activities at Los Alamos, it was necessary to develop a rapid method, more accurate than available colorimetric methods, for determination of acid-soluble sulfur on the microgram scale.

The method is presumably applicable to all materials that are soluble in hydrochloric acid and release their sulfur as hydrogen sulfide. As given, it is applicable to the microgram range, but may be used for determining milligram amounts of sulfur if 0.1 N solutions are employed.

The successful application of the modified standard distillation method to the microgram scale, despite the fact that the small amount of sulfur is determined by difference, depends primarily on two factors: oxidation of the sulfide to sulfate rather than to sulfur as in the usual procedure $S^{--} + 4OCl^- \longrightarrow SO_4^{--} + 4Cl^-$ and the inherent accuracy of the iodometric end point.

Unfortunately, stoichiometric relationships are not borne out experimentally. The reason for this is not known at present. Quantitative results are obtained by determining the titer values of the reagents against known amounts of sulfide.

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PRINCIPLE OF METHOD

The basic reactions involved are:

$$S^{--} + 4OCl^- \longrightarrow SO_4^{--} + 4Cl^-$$

$$OCl^- + 2I^- + 2H^+ \longrightarrow I_2 + H_2O + Cl^-$$

$$I_2 + 2S_2O_3^{--} \longrightarrow 2I^- + S_4O_6^{--}$$

REAGENTS AND APPARATUS

Calcium Hypochlorite (*1*, *2*). Dissolve 6 to 10 grams of U.S.P. calcium hypochlorite, depending on the chlorine content, in 250 ml. of distilled water, shake well, and filter. Dilute the filtrate to 1 liter and store in an amber bottle in the dark. Under these conditions, the solution is stable. This solution is approximately 0.1 N. For use on the microgram scale, dilute to 0.01 N each day before use and redetermine the titer.

A procedure for determining acid-soluble sulfide on the microgram scale is presented. The sulfur is distilled as hydrogen sulfide from acid solution and absorbed in a measured excess of calcium hypochlorite. The sulfide is oxidized to sulfate and the excess hypochlorite determined iodometrically. Stoichiometric relationships are not borne out experimentally. Quantitative results are obtained by determining titer values of reagents against known quantities of sulfide.

Potassium Iodide, 0.1 *N*.
Sodium Thiosulfate, 0.1 *N*. Dilute to 0.01 *N* for use.
Starch Indicator Solution.
Standard Sodium Sulfide Solution. Prepare a stock solution containing 1 mg. per ml. If the large clear crystals of sodium sulfide are hand-picked for weighing, the calculated value will closely approximate the standardization value. Standardize the stock solution against standard iodine–potassium iodide and sodium thiosulfate. Dilute to desired range before use, checking the standardization of the stock solution immediately before dilution.
Pyrex Still (Figure 1).
1-Ml. Buret, calibrated in hundredths.

PROCEDURE

Determine the titer by adding 100 micrograms of sulfide sulfur to 2 ml. of 0.01 *N* hypochlorite in a 25-ml. Erlenmeyer flask. This is done conveniently by employing a calibrated 100-microliter syringe pipet. After a minute or so, add 2 ml. of 0.1 *N* potassium iodide followed by 2 drops of concentrated sulfuric acid. Allow the reaction to proceed for 5 minutes, so that the iodine reaction will go to completion. Titrate the liberated iodine with 0.01 *N* thiosulfate, adding 2 drops of starch indicator just before the end point.

Denote this volume of thiosulfate as a. Similarly, determine the volume of thiosulfate required to titrate 2 ml. of 0.01 *N* hypochlorite without added sulfide, A. Then, the titer $K = \frac{100\ \gamma}{A - a}$.

Introduce the weighed sample into the distilling flask and assemble the apparatus. Pipet 1 ml. of 0.01 *N* hypochlorite into the 25-ml. receiving flask and add a little distilled water, so that the tip of the condenser tube is below the surface of the hypochlorite solution. Add sufficient hydrochloric acid through the side arm, A, to effect solution of the sample. Wash the acid down with a small amount of water and immediately connect the air stream. Adjust to a moderate rate, so that the flow is rapid enough to prevent sucking back when the still cools. Heat the solution in the flask to 80° to 90° C. Best results are obtained if the solution is not boiled. Sweep for 5 minutes, longer sweeping time increases the blank value. It was found experimentally that all the hydrogen sulfide is carried over in 5 minutes. It is important to be sure that the sample is in solution before starting the sweep interval.

Table I. Recoveries of Known Amounts of Sulfide without Distillation

S^{--} Taken, Micrograms	S^{--} Found, Micrograms	%
1000[a]	1010	101.0
100.0	100.0	100.0
50.0	50.0	100.0
20.0	20.1	100.5
5.0	6.3	126.0

[a] 0.1 *N* reagents employed.

At the conclusion of the determination, lower the receiving flask, disconnect the tip, and wash inside and out into the receiving flask with a small quantity of water.

Add 2 ml. of 0.1 *N* potassium iodide and 2 drops of concentrated sulfuric acid and mix thoroughly. Allow 5 minutes for the iodine reaction to go to completion and titrate with 0.01 *N* thiosulfate, adding 2 drops of starch solution just before the end point. Determine the blank by running through the distillation procedure without added sulfide.

If m ml. of thiosulfate are used to titrate the residual hypochlorite, then

$$\gamma\ S^{--} = (B - m)K$$

where B is the volume of thiosulfate required to titrate the 1 ml. of 0.01 *N* hypochlorite carried through the blank determination.

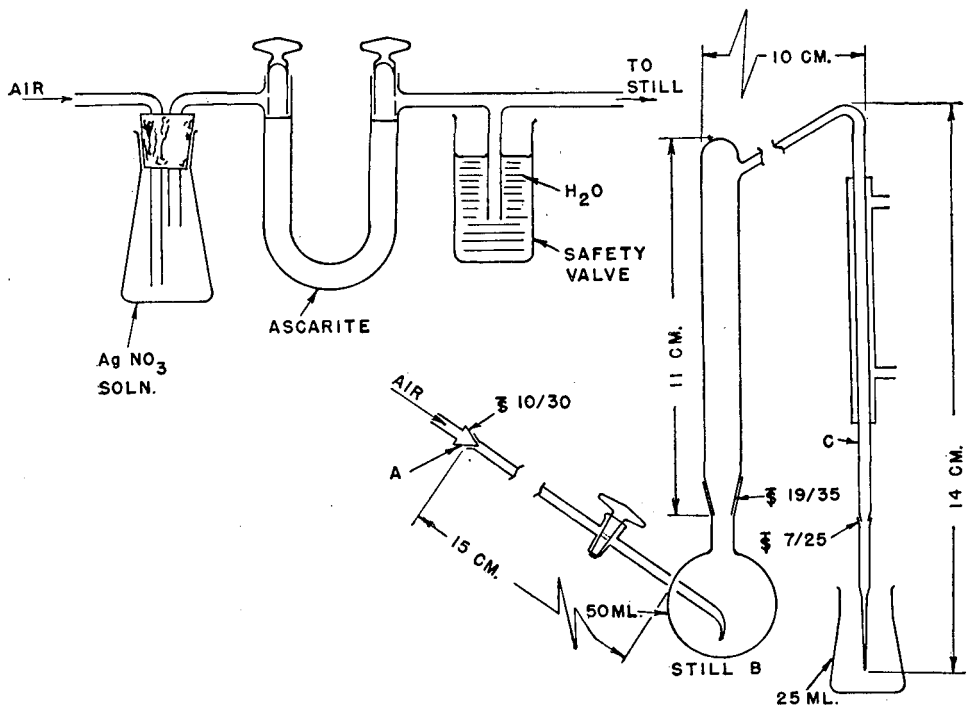

Figure 1. Pyrex Distillation Apparatus

DISCUSSION

Table 1 gives some typical recoveries of known amounts of sulfide added directly to 0.01 *N* calcium hypochlorite. The values reported are individual values and not averages.

Table II presents the recovery of known amounts of sulfide carried through the complete procedure including the distillation.

In order to effect a complete distillation at the microgram level, it is necessary to sweep out the hydrogen sulfide from the

Table II. Recoveries of Known Amounts of Sulfide Including Distillation

S^{--} Taken, Micrograms	S^{--} Found, Micrograms	%
50.0	49.0	98.0
20.0	19.2	96.0
5.0	5.1	102.0
1.0	0.9	90.0

warm solution with a stream of air. Air from the usual compressed air line contains relatively large amounts of sulfur, which is removed by bubbling the air through the silver nitrate solution. A simple and very effective way to prevent the air stream from carrying over any entrained silver nitrate is to back up the silver nitrate scrubbing flask with a standard U-tube filled with Ascarite. This arrangement, plus a simple safety valve (Figure 1), proved extremely effective. No trace of sulfide or silver ion could be detected in the air stream.

The small blank correction is necessary if the distillation is performed. The blank is positive and constant and is apparently caused by the destruction of a small quantity of hypochlorite during the distillation. No blank correction is necessary if the sulfide is added directly to the hypochlorite.

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Spectrochemical Determination of Beryllium

Increased Sensitivity of Detection in the Cathode Layer

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A spectrochemical method for the determination of beryllium in biological material (*1*) has been revised to take advantage of the increased sensitivity of detection made possible by volatilization from the cathode of the direct current arc. In quantitative procedures the method is sensitive to 0.001 microgram of beryllium in the arc, while as little as 0.00025 microgram can be detected in unweakened spectra. The most useful analytical range, employing the beryllium line at 2348.6 Å. extends from 0.005 to 0.30 microgram per ml. of prepared solution. The method described earlier is used to supplement the present method within the range of 0.2 to 2 micrograms beryllium per ml., and a separate working graph for the line pair beryllium 2650.8 Å., thallium 2580.2 Å. is used for the range 2.0 to 30 micrograms of beryllium per ml. of solution.

THE determination of beryllium in biological material by a spectrochemical method (*1*) has proved feasible in the case of samples in which the absolute quantity of beryllium present in the arc is not less than 0.02 microgram. Smaller quantities have been detected frequently, but, in general, detection was not certain when the quantity of beryllium was below the value cited above. Failure to detect beryllium with certainty by this method, in the large proportion of the available samples of the urine of persons known to have been exposed to dusts and vapors of beryllium and its compounds, has indicated that the concentration of beryllium in the urine of such persons is commonly very small. If analysis of the urine is to be a useful tool for estimating the extent of individual or group exposure to beryllium compounds, it must be possible to detect a quantity on the electrode equivalent to 0.01 microgram in the original aliquot of 50 ml. of urine taken for analysis. Actually, a spectrographic method possessing a sensitivity of 0.001 microgram of beryllium or less in the arc would be desirable, for then, on isolating and concentrating beryllium by the procedure outlined previously (*1*), it would be possible to detect as little as 0.1 microgram of beryllium per liter of urine.

A remarkable increase in the sensitivity of the spectrographic detection of beryllium is obtained when the sample is volatilized from the negative electrode of the direct current arc (*2*). This type of excitation has made it possible to detect with certainty somewhat less than 0.001 microgram of beryllium in the arc, and has led to the development of a spectrochemical method particularly adapted to the determination of very small quantities of beryllium. This paper is confined to a description of the changes introduced into the spectrographic procedure, and to a discussion of the factors that influence the ultimate sensitivity of detection and the accuracy of analysis.

PROCEDURE

Spectroscopic Buffer Solution. Fifteen milliliters of a stock solution simulating the salt content of urine (*1*) are mixed with 50 ml. of hydrochloric acid (specific gravity 1.19) and 5 ml. of thallic nitrate solution (1 ml. = 5 mg. of thallium), and the whole is diluted to 500 ml.

Preparation of Samples. Samples are prepared in exact accordance with methods described previously (*1*), except that the weaker spectroscopic buffer solution, described in the preceding paragraph, is substituted for that described in the earlier paper.

Spectroscopic Procedure. Portions of 0.2 ml. of the prepared solutions are placed in the craters of each of two graphite rods (0.63 cm. in diameter and 3.75 cm. in length, with craters 3 mm. in diameter and 10 mm. in depth). The impregnated rods are dried in an oven at 110° C. and each is used as the lower (negative) electrode of the arc. The upper, positive electrode consists of an unimpregnated rod of similar shape and size. The arc is operated from a 110 volt direct current power main, with sufficient ballast in the line to draw 10 amperes. A constant arc gap of 5 mm. is obtained by maintaining the projected image of the arc between two properly spaced marks on a screen. The light of the arc is so focused on the slit of the spectrograph that the glowing "cathode layer" just enters the top of the slit, while light from the incandescent anode is prevented from entering the instrument by the housing of the slit. [The cathode layer may also be focused on the collimator lens of the spectrograph by means of the system of convex lenses, screens, and diaphragms described by Strock (*4*).] The width and length of the slit are the same as those indicated previously, and the rotating step sector is also used as before (*1*). Eastman No. 33 plates are used to photograph the spectra during

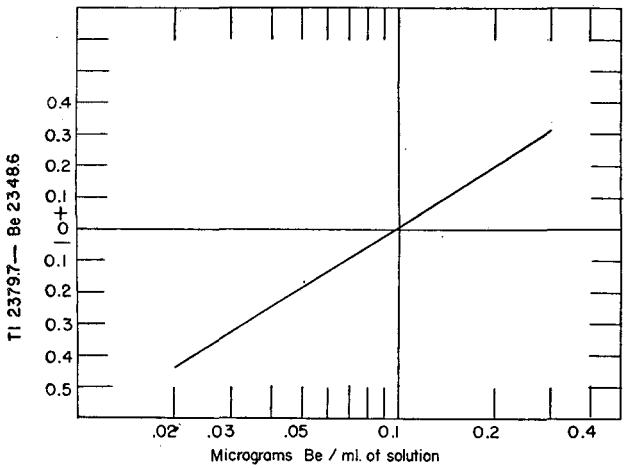

Figure 1. Working Graph for Log Separation Method

2 minutes of exposure. Working graphs obtained by the conventional method of "log I ratios," and also by the use of separations of the blackening curves, are illustrated in Figures 1 and 2. In the case of the former method (Figure 2), the intensities of the lines are corrected for background by the method of Pierce and Nachtrieb (*3*).

Table I. Recoveries of Beryllium Added to Samples of Urine

(50 ml.)

Beryllium Added, γ	Beryllium Recovered, γ
0	Nil, Nil
0.02	0.01, 0.01, 0.02
0.04	0.02, 0.02, 0.03
0.08	0.065, 0.07
0.16	0.17, 0.15

Table II. Typical Analyses of Material by Cathode Layer Method

Material	Size of Sample	Be, γ
Urine (48-835)	50 ml.	0.025; 0.01
Urine (47-7100)	50 ml.	Nil or <0.005
Urine (48-707)	50 ml.	0.095; 0.09
Blood (48-469)	51 grams	Nil or <0.005
Kidney stone (48-1054)	11 mg.	0.035
Kidney stone (48-472)	69 mg.	Nil or <0.005
Lung (47-8028)	18.3 grams	Nil or <0.005
Formaldehyde (47-8024)	200 ml.	Nil or <0.005
Air sample[a]	87.5 liters	Nil or <0.005
Heart, rabbit (48-361)[b]	6.5 grams	0.10
Urine, rabbit (48-1028)[b]	10.9 ml.	0.10

[a] At outlet of "precipitron" in efficiency test.
[b] Experimental animal exposed to beryllium.

RESULTS

Analytical recoveries obtained by this modified method are indicated in Table I, which lists the results of the analysis of duplicate 50-ml. samples of urine, to which known amounts of beryllium had been added. Table II lists some typical data obtained in relation to various samples from persons and experimental animals exposed in varying manner and degree to pulverized beryllium compounds.

DISCUSSION

When the most sensitive line of the spectrum excited by the arc (2348.6 Å.) is used, the range of satisfactory analysis lies between 0.005 and 0.30 microgram of beryllium per ml. of solution (0.001 to 0.06 microgram on the arc). The upper limit can be extended by calibrating a number of working curves for other less sensitive lines, but it has been found more convenient to work within this range only, and to supplement the method with two higher ranges obtained with the conventional direct current arc (*1*). Accordingly, a routine procedure is followed which reduces the need for repetitive analyses to a minimum. This scheme is based on analytical experience which has demonstrated that, as a rule, samples of urine, blood, and most tissues other than lung need to be analyzed by volatilization from the cathode. Samples of lung and of contaminated air, and large samples of certain tissues of exposed experimental animals (liver, kidney) can generally be analyzed by the method described earlier (*1*). An additional working curve has also been developed for the line pair Be 2650.8 Å. and Tl 2580.2 Å. to extend the analytical range to 30 micrograms per ml. of solution (6 micrograms on the arc) and so to avoid the necessity of repeating analyses within this range.

Occasionally, a sample does not fit into the above scheme. In such cases, if it is recognized, following volatilization from the cathode, that the concentration of beryllium is beyond 0.3 microgram per ml. of solution, the residuum of the prepared solution of the sample is diluted with an equal quantity of a spectroscopic buffer (*1*), which contains 188 ml. of the urine salt base and 75 mg. of thallium in 500 ml. of 10% hydrochloric acid. The analysis is then repeated by volatilizing from the anode and applying the working curves derived for this type of excitation (*1*). On the other hand, if the use of anodic excitation, in the first instance, has indicated the presence of a concentration of beryllium below 0.1 microgram per ml., the sample should be diluted with an equal volume of distilled water and the analysis repeated by resorting to volatilization from the cathode.

The intensity of a spectral line in the cathode layer is affected by a number of factors, and satisfactory results can be obtained only by operating under strictly standardized conditions. The same portion of the cathode layer must be photographed each time, and therefore the size of the arc gap must be maintained constant during the period of its burning. The focusing of the cathode layer on the slit or collimator must be done carefully and checked constantly.

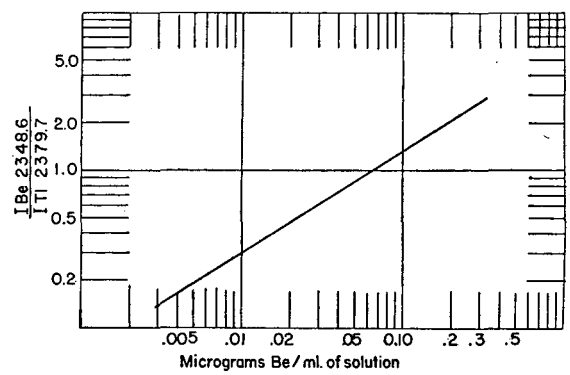

Figure 2. Working Graph for Log I Ratio Method

The greatest sensitivity of detection from the cathode layer has been obtained when only 1 to 3 mg. of materials are present in the arc. Overloading the arc destroys the cathode layer effect (*2*), and this factor offers the greatest difficulty when the method is applied to biological material.

Although the method of isolating beryllium eliminates ions other than those that precipitate as the phosphate, the amounts of the latter are so variable in many biological materials (in urine, for instance) that it is impossible to obtain solutions that are constant in composition and quantity of salts from successive samples of the same type. As a result it is necessary to compromise by employing conditions that give consistent results without a maximum of sensitivity. The strength of the spectroscopic buffer employed as the diluent for such solutions is such

that it contributes approximately 10 mg. to the quantity of salt otherwise available in the arc. The total salt content present in the arc will then be determined by the amount of phosphate precipitated in isolating the beryllium from a sample, so that, on occasion, the arc load may amount to 15 or even 20 mg. of salt. Under these conditions, there is a definite decrease in line intensity, yet the decrease is not sufficient to cause the disappearance of the beryllium line at 2348 Å., even with as little as 0.001 microgram of beryllium in the arc. Moreover, the decrease in intensity due to increased loading of the arc is not harmful from the quantitative standpoint, inasmuch as apparently the intensity ratios of the line pair (beryllium 2348 Å. to thallium 2398 Å.) is affected only slightly if the total load of salt is not in excess of 20 mg.

In order not to increase the amount of phosphate obtained in isolating and concentrating beryllium, calcium phosphate is not added to samples that yield a satisfactory precipitate when neutralized with ammonium hydroxide. In general, such additions are unnecessary except in the case of very small quantities of tissue or samples of urine of extremely low specific gravity. In these instances, the addition of 2 to 3 drops of calcium phosphate solution (*1*) will produce a sufficient quantity of precipitate to entrain all the beryllium in the sample.

The analytical error, as can be seen from Table I, is greater at the lower than at the upper analytical limit. Nevertheless, in view of the minuteness of the quantities of beryllium involved and the difficulties of measuring the intensities of weak lines, the error is acceptable, particularly as, in this range, specificity and sensitivity of detection are more important than absolute accuracy. In so far as sensitivity of detection is concerned, there is no difficulty in detecting as little as 0.001 microgram of beryllium in the arc. This figure, in fact, is on the conservative side, as it is based on the visibility of the line only in the step of maximum exposure of the stepped spectrum. Because each spectrum contains a portion of spectrum corresponding to the length of slit not covered by the rotating sector, a section of spectrum is always present in which the intensity of the beryllium line has not been weakened by the sector. By inspecting this portion of the spectrogram one can tell whether beryllium is present when its presence in the sector-weakened step is questionable. Observation of the unreduced section of spectrum will serve, generally, to reveal the presence of 0.00025 microgram in the arc.

In connection with methods of densitometry, calibration graphs are shown in which data based upon both separation of blackening curves and log I ratios are employed. The latter procedure is preferred, particularly for the lower limits of concentration, because in this range the beryllium line may appear only in the step of maximum exposure, and therefore it will not be possible to plot a blackening curve for the line.

In using a method of such extreme sensitivity, the problem of contamination becomes a consideration of major importance. Although beryllium occurs rarely, if at all, as a natural constituent of biological material or in the reagents used for its determination, misleading results may be obtained unless the analyst is always on guard to prevent contamination. Experience has shown that it is best to use two separate sets of glassware, one set exclusively for preparing samples of high beryllium content and the other for preparing those of low content. A piece of glassware from the first set, although scrupulously cleaned with acid, may contribute a trace of beryllium to a sample that is to be analyzed by volatilization from the cathode. It is also good practice, when sufficient material is available, to analyze samples in duplicate in order to detect adventitious contamination. Beryllium dust may be introduced into the laboratory on the hands, shoes, or clothing of persons who come in contact with such dusts, whether they are engaged in production or in any type of experimental work, and such individuals, and all their operations and equipment, must be excluded entirely from the analytical laboratory. These precautionary measures have been found essential only after costly experience, and the analyst is advised to observe them if he expects to obtain reliable analytical results.

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Determination of Oil in Petroleum Waxes

Rapid Semimicromethod

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THE present standard method for the determination of oil content of paraffin wax has the A.S.T.M. designation D721–47 (*1*). The procedure involves dissolving 25 grams of wax in 375 ml. of methyl ethyl ketone, precipitating the wax by cooling to −25° F. filtering, and determining the oil in an aliquot of the filtrate by weighing the residue after evaporation (*1*, *2*). Although D721–47 yields reliable results and is satisfactory as a referee procedure, it has two important disadvantages when considered as a refinery control test:

Approximately 6 hours are required to complete a determination. In sweating, blending, and other wax processing operations, where it is important to maintain a control of the oil content, the time required for a determination is excessive. The need for a rapid accurate procedure is obvious for such purposes.

The equipment required for performance of D721–47 is bulky and cumbersome. Considerable bench space is required and in practically all petroleum laboratories such space is held at a premium.

It was felt by the authors that the present macroprocedure is ideally suited to reduction to a semimicro scale, and that this reduction would afford significant savings in time, space requirement, and cost of performance.

At the outset, the following three basic factors were established as conditions which this semimicromethod should meet, if it is to receive wide acceptance in the petroleum industry.

Results should agree with those of D721–47 and be at least as precise. All the essential features of D721–47 should be retained. This seemed important in view of the fact that the distinction between "oil" and "wax" is one of definition, as no sharp physical or chemical boundary exists.

The method should be readily adaptable for use in control laboratories and not require specialized equipment such as a microbalance.

A nontechnical operator, after having been trained properly in the method, should be capable of obtaining satisfactory results.

A rapid and accurate semimicromethod for the determination of oil in petroleum waxes has been developed. All the essentials of the standard method of test for oil content of paraffin wax, A.S.T.M. designation D721–47, are retained but the sample size is reduced to 1 gram. Results by the two methods agree closely. An ordinary analytical balance is used and no special training on the part of the operator is required. Savings are effected in solvents, bench space, and cooling capacity. A determination may be made within an hour as contrasted with approximately 6 hours for D721–47. This greatly increased speed should be particularly welcomed by petroleum refinery laboratories where manufacturing control in wax processing operations calls for quick determinations. By variation of the solvent, solvent ratio, and temperature, the procedure may be applied to microcrystalline waxes.

The most time-consuming step in D721–47 is the evaporation of about 100 ml. of solvent from the oil residue. The possibility of shortening this step by a semimicroprocedure was obvious. By scaling down to $^1/_{25}$th the amount of wax and methyl ethyl ketone taken and designing apparatus to suit the smaller quantities, the evaporation actually can be completed in less than 0.5 hour as contrasted with approximately 3.5 to 4 hours in the instance of D721–47.

A number of other steps are simplified and accelerated. Chilling is more rapid and a specially designed copper vessel becomes unnecessary. Dissolving and chilling are performed in a glass test tube and the transfer of the solution with its precautions is entirely eliminated. The solvent can be measured with a pipet, which eliminates one weighing. Because the amount of solvent is greatly reduced there is no need for its recovery. One batch of solvent will last for a long time, which means that blanks need be determined less often. Finally, the apparatus required for the semimicroprocedure is simple and compact and requires very little space.

No method for the determination of oil in microcrystalline wax has been standardized by the A.S.T.M., although several methods are being used by petroleum refiners. The results by these procedures are purely empirical in that they are entirely dependent upon such factors as the nature of the solvent used, wax-solvent ratio, extraction temperature, and other variables. The Socony-Vacuum Laboratories have developed and are now using a method which consists of dissolving the microcrystalline wax in ethylene dichloride, cooling to 0° F. for the precipitation of the wax, filtering off an aliquot portion of the ethylene dichloride containing

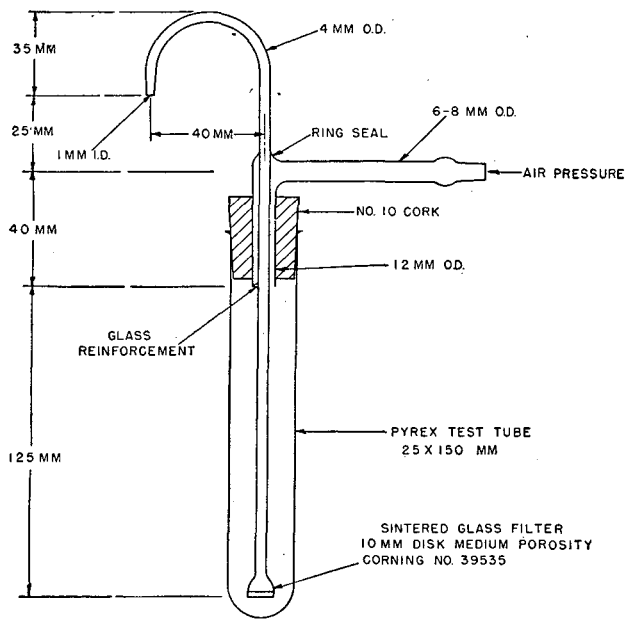

Figure 1. Filter Stick and Assembly

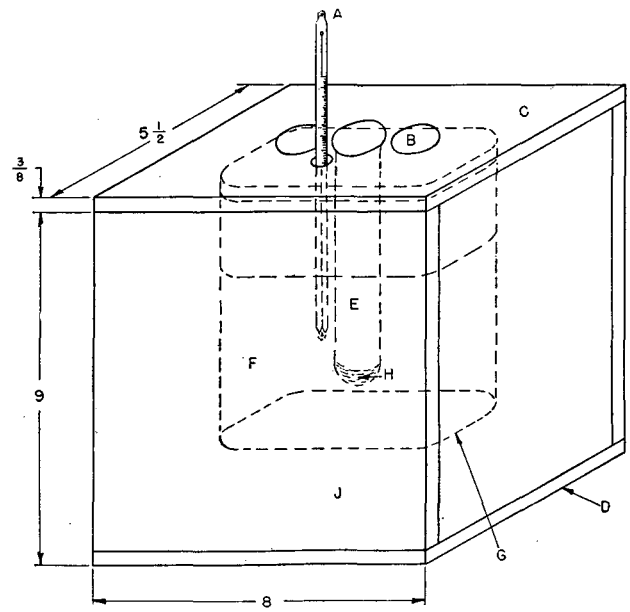

Figure 2. Cooling Bath

A. **Thermometer, A.S.T.M. low cloud and pour**
B. **1-inch hole**
C. **Bakelite top**
D. **Wood box, 0.375-inch plywood**
E. **Test tube, 25 × 150 mm.**
F. **Kerosene and dry ice**
G. **1-quart metal can**
H. **Absorbent cotton**
J. **Glass wool insulation surrounding can**

dissolved oil, and determining the oil content of this filtrate by evaporating the solvent and weighing the residue. The apparatus required for the method is identical to that used in D721–47. The semimicrotechnique has been applied with the same success as in the instance of paraffin wax, and the apparatus and techniques are almost identical.

APPARATUS

Assembly for filtration under pressure with sintered-glass filter stick. The dimensions are given in Figure 1. This is connected with rubber tubing to the pressure bottle as indicated.

Pyrex test tubes, 25 × 150 mm., selected to pass easily through the 2.5-cm. (1-inch) hole in the cooling bath top. Test tubes should be permanently equipped with wire loops for suspending them from the hook of the balance during weighings.

Cooling box, an insulated bath with 2.5-cm. (1-inch) holes in the cover to accommodate the test tubes. It should contain sufficient kerosene (pour point below −50° F.) to cover the contents of the test tubes during chilling. It is cooled by the addition of granulated dry ice or circulation of a coolant through coils. A cooling box of suitable dimensions is shown in Figure 2.

Pressure bottle, a heavy-walled glass filter flask with two-holed stopper and stopcock for the control of pressure (Figure 3).

Dropper pipets. For paraffin wax, dropper pipet with rubber bulb calibrated to deliver 1 ± 0.05 gram of molten wax (Figure 3). For microcrystalline wax, a similar pipet calibrated to deliver 0.5 ± 0.025 gram of molten wax.

Transfer pipets. For paraffin wax, a transfer pipet to deliver 15 ml. of methyl ethyl ketone. For microcrystalline wax, a transfer pipet to deliver 20 ml. of ethylene dichloride. For this a suction line or bulb should be used and not the mouth.

Two thermometers, A.S.T.M. low cloud and pour −70° to +70° F. It is convenient to place additional markings with ceramic ink on one thermometer to indicate the cooling bath temperature ranges (−30° to −35° F. for paraffin and −5° to −10° F. for microcrystalline wax) and on the other, the filtration temperatures (−25° and 0° F., respectively).

Glass-stoppered conical weighing bottles, capacity 15 ml.

Rubber-tipped forceps for handling weighing bottles.

Evaporation assembly consisting of an evaporating cabinet and connections similar to that in Figure 4. The temperature in the cabinet is maintained at about 95° F., preferably by means of a thermostat. Jets are provided with an inside diameter of 4 ± 0.2 mm. for delivering a stream of clean dry air vertically downward into the evaporation flasks. They are supported so that the tip of each jet may be set 15 ± 5 mm. above the surface of the liquid at the start of the evaporation. The size of the evaporating assembly and the number of jets are optional. The air is purified by passage through a tube of 1-cm. bore packed loosely for 20 cm. with absorbent cotton. This has proved adequate at the Socony-Vacuum Laboratories, although others may prefer to retain the 30-cm. column of fuller's earth as described by the A.S.T.M. (*1*). It is absolutely imperative that blanks be run at frequent intervals to check on the cleanliness of the air and absence of residue in the solvent.

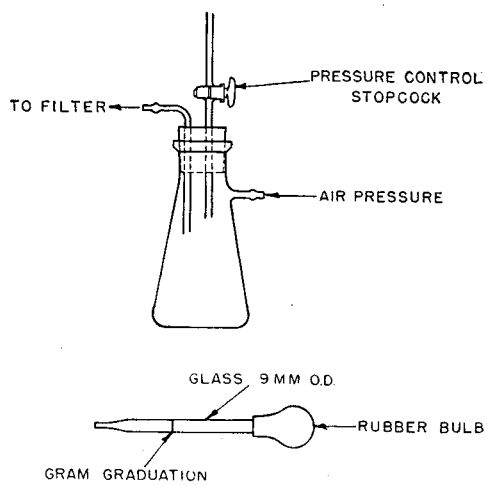

Figure 3. Pressure Bottle and Wax Pipet

An analytical balance capable of reproducing weights to about ±0.05 mg. The sensitivity of the balance should be tested and, if necessary, adjusted to assure the required precision of weighing.

All test tubes, weighing bottles, and caps should be numbered with ceramic ink or other permanent markings to prevent confusion.

SOLVENTS

For paraffin wax. Methyl ethyl ketone, commercial grade, refractive index at 68° F. 1.378 ± 0.002. The residue left after evaporation of 4 ml. according to the procedure described below should not exceed 0.1 mg.

For microcrystalline wax. Ethylene dichloride, recently distilled technical grade boiling between 176° and 187° F. The residue left after evaporating 5 ml. should not exceed 0.1 mg.

PROCEDURE

Preparation of Apparatus for Use. Test tubes should be cleaned after each determination by pouring out the remaining wax and solvent after heating to obtain a clear solution, and washing with xylene while still warm. They should be allowed to drain or be wiped dry. The weight of these tubes when cleaned will not vary significantly, so that a tare weight may be obtained once and be used repeatedly. Two tare flasks are convenient; one for weighing test tubes, and the other for weighing the weighing bottles.

The weighing bottles should be cleaned out with a few milliliters of xylene followed by methyl ethyl ketone. After this solvent has been poured out, they should be carefully wiped off on the outside and dried in the evaporator. Once they have been dried, they should be lifted only with forceps. The operator's hands must be clean and caps should be removed and replaced with a light touch. Slight fluctuations in the initial weight, from one analysis to the next, of a few tenths of a milligram are to be expected. For this reason, it has not been possible to dispense with a weighing of the empty bottle for each determination when waxes of low oil content are being analyzed. For waxes containing several per cent of oil, this variation is not serious enough to warrant checking the weight of the empty bottles.

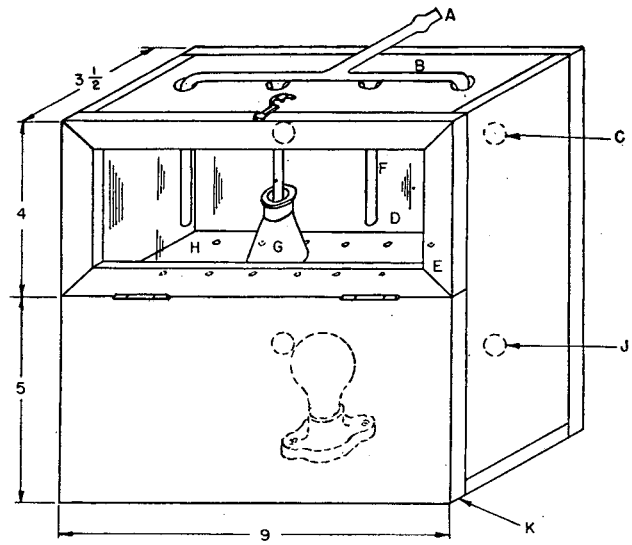

Figure 4. Evaporator

A. **Filtered air from flowmeter**
B. **Pyrex manifold, 6 mm. in outside diameter**
C. **Air exit, 0.75-inch diameter**
D. **Glass door**
E. **Wood door frame**
F. **Jet, 4 mm. in inside diameter**
G. **15-ml. weighing bottle**
H. **Aluminum platform with holes for hot air, 0.25 inch in diameter**
J. **Air entrance, 0.75-inch diameter**
K. **0.375-inch plywood**

The filter stick is cleaned out by pouring a few milliliters of methyl ethyl ketone into a clean extra test tube, connecting for filtration, and blowing through with air pressure until the air drives the solvent from the tube. Because of capillarity, the filter disk retains liquid tenaciously. This last liquid may be most effectively removed by holding against the disk a piece of absorbent cotton which will draw nearly all of the liquid from the disk. The filtration assembly is inserted into a clean, dry test tube, and the filter is blown completely dry.

If dry ice is used as the coolant, it must be crushed to a coarse snow. Introduction of large fragments of dry ice into the kerosene bath will result in large bubbles and overflowing. Moreover, too rapid addition of dry ice will cause foaming and overflowing. The small size of the cooling bath suggests the feasibility of improving the equipment in the future by designing an electrically refrigerated thermostated unit.

Some crushed ice is mixed with water in an 800-ml. beaker.

The bulb in the evaporator is turned on to warm up to evaporating conditions. Air is passed through at 2 to 3 liters per minute per jet.

Determination of Oil in Paraffin Wax. A representative sample of the wax is melted in a beaker at a low temperature, as on a steam plate, to minimize oxidation. If possible, the dropping pipet is heated simultaneously in the wax.

The wax is thoroughly mixed by stirring as soon as it is completely melted and the sample is taken with the dropper pipet without delay. The pipet held vertically is inserted in the test tube and the wax expelled without spattering any on the sides. The pipet is carefully withdrawn and the test tube is swirled to coat the bottom evenly with wax. This permits more rapid dissolving later. The test tube is cooled, in air if a series of samples is being taken, or in running water if a single determination is performed and maximum speed is essential. The test tube is then weighed to the nearest milligram. The companion weighing bottle which has truly acquired room temperature by standing for 10 minutes in the balance room, is weighed to the nearest 0.1 mg.

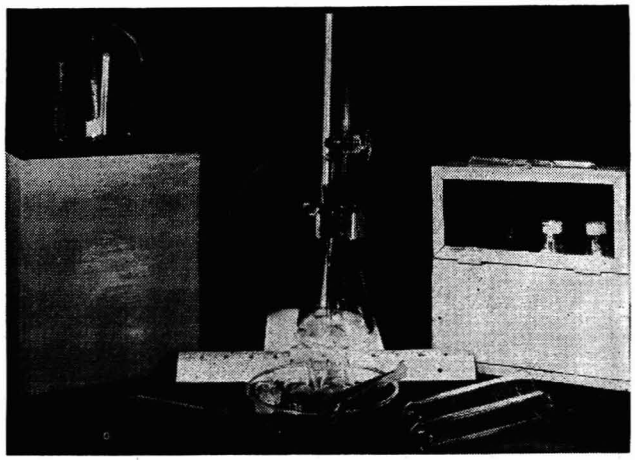

Figure 5. General View of Socony-Vacuum Semimicroapparatus for Determining Oil in Petroleum Waxes

A significantly low weight would be obtained if the temperature of this bottle were even slightly above room temperature.

Fifteen milliliters of methyl ethyl ketone are pipetted into the test tube, which is then inserted, just up to the level of its contents, into a steam bath. The solvent will boil and reflux from the sides of the tube. As soon as the wax is completely in solution, the tube is removed and plunged into the 800-ml. beaker of ice water. The loss of solvent through vaporization is less than 1%. The weight of the solvent is, therefore, practically a constant, and after a few samples are weighed, this weight can be used as a constant factor. It will be about 11.9 grams, one decimal being sufficient.

The temperature of the cooling bath is adjusted to be in the range from $-30°$ to $-35°$ F. The filter is kept in the middle test tube, so as to acquire the temperature of the bath. Clean absorbent cotton is placed at the bottom of this test tube to suck the filter stick dry between determinations. It is only necessary to let the filter drain by gravity, wipe off the outside of the filter with cotton to remove wax, and replace in this test tube after each filtration.

For routine series determinations on waxes of like oil content, this treatment removes all but traces of the previous solution and does not introduce a serious error. However, significant error can result if the oil content of successive waxes varies greatly. In such instances, it will be found advisable to wash out the filter with methyl ethyl ketone and dry it as described under preparation of apparatus for use.

The test tube containing the sample is removed from the ice bath, wiped dry on the outside, and inserted into the cooling bath through one of the vacant holes. The mush of wax and solvent is stirred with the thermometer, scraping the sides and bottom, until the temperature reads $-25° \pm 0.5°$ F. The thermometer is removed. The precooled filter assembly is now introduced into the test tube with the cooled sample. The stopper is pressed firmly into the sample test tube so as to make an air-tight seal. The weighing bottle is unstoppered and placed under the delivery nozzle of the filter assembly. The weighing bottle is best placed on a cork, to keep it off the Bakelite top of the cooling bath which sometimes becomes wet with kerosene. The stopper of the filter assembly is held down with the left hand, while the right turns on the air pressure, the stopcock of the pressure bottle being open. After a little experience, the pressure can be gaged by the hiss of air escaping through the stopcock. The stopcock is turned, but not fully closed, until the liquid rises in the filter tube and drops steadily into the weighing bottle.

When about 4 ml. have been filtered off, the air pressure is turned off, which will permit the filter to drain back slowly. The weighing bottle is meanwhile removed, stoppered, and immediately weighed to the nearest centigram without waiting for it to come to room temperature. It is then unstoppered and placed under one of the jets in the evaporator. After the solvent has evaporated, which usually takes less than 30 minutes, the bottle is removed, stoppered, allowed to acquire room temperature, and weighed to the nearest 0.1 mg. It is then returned to the evaporator for about 5 minutes and weighed so as to confirm completion of the removal of solvent.

The oil content is calculated with the use of the equation:

$$\% \text{ oil} = \left(100 \times \frac{\text{weight of solvent} \times \text{weight of oil}}{\text{weight of sample} \times \text{weight of solvent evaporated}}\right) - 0.15$$

The factor 0.15 corrects for the amount of wax soluble in methyl ethyl ketone at $-25°$ F. Results less than zero are reported as zero.

It is important to keep in mind the precision required of each weighing. The weights of the weighing bottle, empty and containing the final oil residue, limit the precision of the method and these weights must be obtained to the nearest 0.1 mg. On the other hand, the test tube with the wax sample is weighed to the nearest milligram (an error of one part per thousand on a 1-gram sample) and the weighing bottle containing filtrate is weighed to the nearest centigram (an error of 1 part in 300 on 4 ml. of solvent.) Therefore, the test tube may be weighed immediately if it has been cooled to within 10° F. of room temperature and the weighing bottle containing filtrate is weighed at once, even though the filtrate within is cold. On humid days, it may be necessary to wipe off condensed moisture before obtaining this latter weight.

Determination of Oil in Microcrystalline Wax. The procedure for microcrystalline waxes is very similar in technique to that described for paraffin waxes.

One-half gram of sample is taken instead of 1 gram. Cooling and weighing are performed as described and then 20 ml. of ethylene dichloride are added. About 24.7 grams of solvents will remain after the sample has been dissolved and cooled. The temperature of the cooling bath is adjusted to the range from $-5°$ to $-10°$ F. The sample is chilled to 0° F. with stirring. Five milliliters of liquid are filtered off, weighed, and evaporated, and the weight of the oil residue is determined.

The percentage of oil is calculated from the equation:

$$\% \text{ oil} = 100 \times \frac{\text{weight of solvent} \times \text{weight of oil}}{\text{weight of sample} \times \text{weight of solvent evaporated}}$$

No correction, such as 0.15 in the case of paraffin waxes, is included in the equation. The oil content is arbitrarily defined as that material which is extracted from the microcrystalline wax by the solvent under the specified test conditions.

Safety Precautions. Both solvents, methyl ethyl ketone and ethylene dichloride, should be considered toxic, particularly if inhaled over long periods. Exposure to too high concentrations of the vapors will produce symptoms of intoxication, headache, and nausea. Ethylene dichloride is known to cause temporary clouding of the cornea; consequently, the apparatus should be confined to a hood wherever continuous work is in progress. However, in view of the small amount of solvent being evaporated, the semimicroprocedure will permit without danger occasional determinations in a large well-ventilated room.

The eyes of the operator should be protected by safety glasses during operations which use hot solvents and during the filtrations under pressure.

EXPERIMENTAL DATA

In order to obtain data on the precision of the Socony-Vacuum semimicromethod on paraffin waxes, six samples were tested, varying from low to high oil content. Triplicate determinations were made by two different nontechnical operators following method D721–47 and by two other operators according to the semimicrotechnique. Operator C (Table I) was an experienced microchemist, whereas operator D was a nontechnical operator with no training in microtechnique.

Table I also shows a similar comparison of the Socony-Vacuum macromethod for oil content determination of microcrystalline wax and the semimicroprocedure applied to such waxes. Four microcrystalline waxes of different oil contents were selected for this comparison, and determinations were performed in triplicate by the same operators who analyzed the paraffin waxes.

Table I. Comparative Oil Content Data on Paraffin and Microcrystalline Waxes

Paraffin Wax Sample	A.S.T.M. Method D721-47						Socony-Vacuum Semimicromethod					
	Operator A			Operator B			Operator C			Operator D		
1	0.11	0.10	0.16	0.11	0.12	0.08	0.11	0.06	0.10	0.14	0.16	0.05
2	0.48	0.46	0.45	0.47	0.52	0.55	0.55	0.51	0.52	0.53	0.49	0.55
3	1.02	1.05	1.26	1.27	1.29	1.08	1.02	1.15	0.99	1.23	1.03	1.26
4	2.6	2.9	2.7	2.6	2.6	2.7	2.9	2.7	2.6	2.7	2.8	2.6
5	7.8	7.0	7.0	7.1	6.8	7.2	7.5	7.5	7.3	7.9	7.4	6.9
6	8.4	8.1	8.7	8.2	7.8	8.0	8.6	8.8	8.4	8.3	9.1	8.3
Microcrystalline Wax Sample	Socony-Vacuum Macromethod						Socony-Vacuum Semimicromethod					
1	3.9	4.4	4.4	3.7	3.4	3.7	3.7	3.7	3.4	3.3	3.2	3.4
2	6.6	7.2	7.0	5.3	6.6	5.3	5.8	5.3	5.7	5.6	5.5	5.0
3	7.8	7.7	8.0	7.4	7.4	7.4	7.4	7.8	7.6	7.5	7.7	7.4
4	12.6	13.4	13.1	10.9	11.1	11.9	11.2	11.2	11.1	10.4	10.7	10.4

Table II. Average Values and Deviations in Test Results

Paraffin Wax Sample	Average Results			Average Deviations	
	A.S.T.M. method D721-47	S.-V. semi-micro	Combined average	A.S.T.M. method D721-47	S.-V. semi-micro
1	0.11	0.10	0.11	0.02	0.03
2	0.49	0.52	0.51	0.04	0.04
3	1.16	1.11	1.14	0.10	0.10
4	2.68	2.72	2.70	0.08	0.08
5	7.15	7.42	7.28	0.32	0.25
6	8.20	8.58	8.39	0.30	0.25
Microcrystalline Wax Sample	S.-V. macro			S.-V. macro	
1	3.92	3.45	3.68	0.32	0.25
2	6.34	5.48	5.91	0.83	0.42
3	7.62	7.57	7.59	0.22	0.13
4	12.2	10.8	11.5	1.0	0.7

Table II shows the average results and the deviations therefrom. It will be seen from these data that, with respect to paraffin waxes, the semimicrotechnique is as reliable as method D721–47, and in the instance of the microcrystalline waxes, the method is as precise as the macrotechnique.

CONCLUSIONS

Oil content determinations by the Socony-Vacuum semimicromethod on paraffin waxes agree closely with results obtained by A.S.T.M. method D721-47; the precision appears to be equally satisfactory. The semimicromethod may be applied to microcrystalline wax with similar success.

The equipment required for the test is simple, inexpensive, and can be readily constructed in a laboratory. The apparatus and technique permit appreciable saving in solvents, bench space, and cooling capacity when compared with method D721-47. The method is excellently adapted for control operations in refinery laboratories.

The most significant advantage of the method is its time-saving feature. After adequate practice, a nontechnical operator should be able to complete a determination in approximately 1 hour. At least 6 hours are generally required for a similar result by method D721–47. The method has the speed of less reliable routine procedures, but appears to have the precision required for a referee method.

ACKNOWLEDGMENTS

The preliminary suggestions as to procedure and apparatus were made by A. A. Benedetti-Pichler, Socony-Vacuum Laboratories consultant, whom the authors wish to thank for valuable advice. The authors also wish to acknowledge the cooperation of D. W. Robertson of their laboratory.

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Microscopical Distinction of Corundum among Its Natural and Artificial Associates

Employing the Christiansen Effect by Transmitted, Dark-Field Illumination

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CORUNDUM, a natural aluminum oxide with a Mohs' scale hardness of 9, is widely used in the grinding of lenses from optical glass. It is found in many parts of the world, but the Transvaal region of South Africa is the source of much of the material imported for use in grinding glass. In processing for use as an industrial abrasive, the imported material is crushed and screened. The grinding wheel industry makes use of the larger screen sizes and the optical industry the smaller sizes for the rough grinding of ophthalmic glass. The subsieve sizes required for grinding precision surfaces are prepared from the material recovered from the rough grinding operation by repeated free settling classification. The cake formed when the water has evaporated is cut by experienced workmen into portions having different average particle sizes.

A control of the purity of the corundum is important at two different points. The sieve size material (60 to 220 grit) purchased for rough grinding is checked with the microscope to see that the amount of quartz, mica, magnetite, etc., is not excessive. The subsieve size fine grinding powders recovered from the rough grinding operations are examined for the above impurities and also for the amount of glass that remains mixed with the corundum. Too much glass will decrease the grinding speed but approximately 15% glass will act as a source of slowly released alkali silicate that will serve as a dispersing agent to prevent agglomeration of the fine corundum into lumps during classification and use.

METHOD

The microscopical inspection of corundum can be made by employment of the Christiansen effect (*1, 2, 3*) by one not trained or equipped for petrographic work. Any microscope having an Abbe condenser of 1.25 N.A. and an achromatic 10× objective

The Christiansen effect is employed to distinguish particles of corundum among its associates in abrasive emery powder either in its natural state or after it has been used to polish glass. This method is especially helpful in the quantitative microscopical estimation of corundum before and after abrasive use, because easy rapid recognition of the corundum among all its associates is obtained with an ordinary compound microscope, without need for petrographic accessories. The method may be extended to any system of phases in which the liquid medium is of favorable refractive index and dispersion, and the relevant determinative properties of significant phases differ sufficiently from one another.

of 0.25 N.A. can be used. The microscope lamp must be very bright, such as a 6-volt 108-watt ribbon filament bulb, and should be equipped with a blue and ground-glass or Daylite filter.

The upper element of the Abbe condenser is removed and a dark-field stop approximately 16 or 17 mm. in diameter is placed in the carrier below the condenser. The abrasive grains are distributed evenly on a glass slide, a drop of methylene iodide is added, and the slide is covered with a thin cover glass. The methylene iodide should be of a refractive index of approximately n_D 1.74 at 25° C. to function satisfactorily with corundum with an "average" refractive index of about 1.77. It is important that the slide and cover glass be clean and free from scratches. The microscope is focused on the preparation and the plane substage mirror is adjusted so that the field is evenly illuminated. The condenser is then racked up or down as may be necessary to make the abrasive grains appear bright against a dark background.

RESULTS

Corundum particles (grain size 500 to 1600), whether natural or synthetic, as seen by this method are golden yellow or yellow with purple or blue borders. Silica, mica, and most types of glass appear white. Mica can be identified as thin flakes showing evidence of basal cleavage, and any iron or magnetite that may be present will be dark and opaque. A sample may be rejected if the proportion of corundum grains is lower than that found in a chemically analyzed sample selected as a standard.

DISCUSSION

The basic method can be used for easy identification of any constituent of a mixture of transparent particles if the other materials in the mixture differ substantially in refractive index, "opacity," color, size, shape, or other convenient property. The immersion liquid must be selected so as to have a refractive index near that of the material to be observed and a much higher dispersion. For an index range of from 1.440 to 1.628, mixtures of diethylene glycol monobutyl ether and Halowax oil (technical grade of α-chloronaphthalene) are suggested. Mixtures of Halowax oil and α-bromonaphthalene can be used for the range between 1.632 and 1.656 and α-bromonaphthalene and methylene iodide from 1.660 to 1.70. Mixtures of diethylene glycol monobutyl ether with cinnamaldehyde giving a range in index from approximately 1.440 to 1.62 are superior to the above for production of strong colors in mounted preparations.

Increased magnification can be attained by the use of higher powered eyepieces with the 10× objective or the use of the achromatic 43× objective of 0.65 N.A. If the latter is used, its numerical aperture must be reduced by the insertion of a funnel stop screwed into the objective far enough so as just to touch the back lens. A funnel stop with over-all length of 2.72 cm. (1.092 inches) and aperture of 2.9 mm. (0.116 inch) has proved satisfactory for most preparations. The size of the dark-field stop located in the carrier below the condenser for use with this objective is approximately 22 mm. in diameter.

Dispersion coloration results from the operation of the same principles of transmittance and refraction of light that govern the action of the Christiansen filter (*1*). If particles of a transparent solid are placed in a liquid having the same refractive index as the solid for one wave length of light but a different refractive index for other wave lengths, the color for which the refractive index of the liquid and solid match will be transmitted without deviation but other wave lengths will be deviated. When a microscope is adjusted for dark-field illumination, only the deviated colors reach the eye.

The method of inspection described gives less information than would result from skilled petrographic or mineralogical microscope study. It is preferable for obtaining approximately quantitative information about a qualitatively familiar system because it saves time, skill, and equipment.

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RECEIVED February 28, 1948.

Standard Samples

The National Bureau of Standards, Washington 25, D. C., has added six new materials to its list of analyzed standard samples.

Standard Silicon-Aluminum alloy, No. 87, has the following percentage composition: silicon 6.22, nickel 0.59, iron 0.46, magnesium 0.39, copper 0.30, manganese 0.30, chromium 0.17, titanium 0.16, zinc 0.075, lead 0.070, tin 0.061. Price, $3.00 per 65-gram unit.

Standard Copper-Nickel Alloy (Monel type), No. 162, has the following percentage composition: nickel 66.38, copper 28.38, manganese 2.34, silicon 0.67, cobalt 0.54, iron 0.34, chromium 0.24, aluminum 0.23, titanium 0.20, carbon 0.11, sulfur 0.002. Price, $3.00 per 150-gram unit.

Standard Cr-Ni-Mo Casting Steel, No. 160, is certified for the following constituents: carbon 0.044, manganese 0.68, phosphorus 0.012, sulfur 0.011, silicon 1.13, copper 0.053, nickel 8.90, chromium 19.13, vanadium 0.038, molybdenum 2.95. Price, $3.00 per 150-gram unit.

Standard Nickel-Chromium Casting Steel, No. 161, is certified for the following constituents: carbon 0.34, manganese 1.29, phosphorus 0.012, sulfur 0.005, silicon 1.56, copper 0.04, nickel 64.3, chromium 16.9, vanadium 0.03, molybdenum 0.005, cobalt 0.47, iron 15.0. Price, $3.00 per 150-gram unit.

Standard Low Carbon, 19 Cr-9 Ni Steel, No. 166, is certified for carbon only, 0.028%. Price, $2.00 per 100-gram unit.

Standard Glass Sand, No. 165, is certified for total iron, as Fe_2O_3 = 0.0189. Price, $2.00 per 65-gram unit.

The bureau now issues more than 400 kinds of standard samples of steels, irons, ferroalloys, nonferrous alloys, ores, ceramic materials, high-purity chemicals for standardizations, hydrocarbons, paint pigments for color, oils for viscometer calibrations, melting-point standards, and other reference standards. A complete list of the standards, fees, and other information is given in the supplement to Circular C398.

NOTES ON ANALYTICAL PROCEDURES . . .

Modified Apparatus for Determination of Groups Active to the Grignard Reagent

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THE so-called Zerevitinov method for the quantitative determination of groups active to the Grignard reagent had its inception in the work of Chugaev (*2*), who noted that many organic compounds, especially those containing hydroxyl groups, produced hydrocarbons when treated with alkylmagnesium halides. Hibbert and Sudborough (*5*) and later Zerevitinov (*13*) took up and expanded the work of Chugaev, applying it to quantitative work.

Although Chugaev himself did not discuss any particular type of apparatus in his original paper, other investigators have described various designs. The apparatus of Hibbert and Sudborough and that of Zerevitinov consisted essentially of a device for mixing the Grignard solution with the solution of the sample in the same glass container and measuring the resulting gas. Other Grignard reactions, notably those involving addition to double-bonded atom groups, could be measured by the apparatus developed by Kohler and his associates (*6, 7*), which was designed to measure both the amount of reagent reacting to form a gas and the total amount of reagent consumed.

Other types of apparatus have been described by Moureu and Mignonac (*9*), Oddo (*10*), Larsen (*8*), Assaf and Gladding (*1*), Evans, Davenport, and Revukas (*3*), and Fuchs, Ishler, and Sandhoff (*4*). Micromethods have been reported by Roth (*11*) and Soltys (*12*).

The apparatus described in this paper involves the principle of Kohler's device, but is constructed of standard pieces of glassware, utilizing standard-taper joints. The original Kohler apparatus was essentially one single piece of equipment, with the exception of the reaction flask. This new device, made of standard items which can be duplicated, permits disassembly for cleaning purposes and easy repair of any broken part.

DESCRIPTION OF THE APPARATUS

E, F, and *L* in Figure 1 are standard "gas inlet" tubes with a 24/40 joint. *A, B,* and *C* are ordinary three- and two-way stopcocks. Flask *D* is of 300-ml. capacity. Buret *K*, for the addition of water, has a capacity of 10 ml., graduated in 0.1 ml., and may be attached to a 24/40 joint or inserted through a tightly fitting rubber stopper. *P* is a standard type mercury-filled gas buret of 100-ml. capacity, graduated in 0.1 ml., with a three-way stopcock, *R*, at the top. Buret *G* has a capacity of 10 ml., graduated in divisions of 0.05 ml. Reaction flask *N* and buret *P* may be jacketed so that water of any desired temperature may be passed around them. The lower portion of *G* is connected to the gas tube, *L*, by a rubber sleeve. The ends of connecting tube *Q* may be sealed directly to *R* and *L* or connected by rubber sleeves. The use of a suitable lubricant is usually advisable to ensure gastight connections, especially of the reaction flask and connecting tube *M*. Broken lines in Figure 1 represent rubber connections which may be substituted.

OPERATION OF THE APPARATUS

Reagents. The Grignard solution was prepared in a typical run from 62.8 grams of methyl iodide, 10.8 grams of magnesium, and 250 ml. of anhydrous butyl ether. Butyl ether was also used as the solvent for the sample to be analyzed. The nitrogen gas was commercial anhydrous nitrogen, which was passed through a calcium chloride drying tube before being passed into the apparatus. (The nitrogen used was supplied by the Air Reduction Company and was of 99.5% purity. If further purification of the gas is desired, it may be passed also through Fieser's solution to remove oxygen; through lead acetate solution to remove traces of hydrogen sulfide; and through sulfuric acid and phosphorus pentoxide, as described by Kohler.) The gage pressure of the nitrogen was maintained at approximately 10 pounds per square inch.

Blank Run and Standardization of Reagent. The apparatus is thoroughly cleaned and dried, and the reagent is filtered through glass wool into flask *D*. Stopcock *A* is opened to the nitrogen source, stopcock *B* is closed, and stopcock *C* is turned to position I (Figure 1) permitting the nitrogen to pass into flask *D*, and creating a pressure therein. *C* is then quickly turned 180° counterclockwise to position II and the pressure relieved by opening *A* to the atmosphere through stopcock *R*, which is turned to bypass the buret. The nitrogen valve is then closed. *B* is opened, allowing the reagent to pass into *G*. When a sufficient amount of the reagent has passed over, the excess remaining in the upper part of the apparatus can be pressed back into *D* by again turning *A* to connect with *C*, which has remained in position II. *B* is then closed, and it remains in this position until it becomes necessary to refill buret *G*.

Five milliliters of the butyl ether solvent are placed in flask *N*, and the flask is replaced. Dry nitrogen is swept into the flask and lower part of the apparatus through stopcock *A* and tube *J*. When the air has been displaced, *A* is closed and *R* is turned to connect with the system. (At this point, it has been found advisable to lower the mercury reservoir below the level of the mercury in the buret to reveal the presence of leaks, if any exist.)

When the level of the mercury in the buret has been read, the reservoir is lowered to create a pressure less than atmospheric

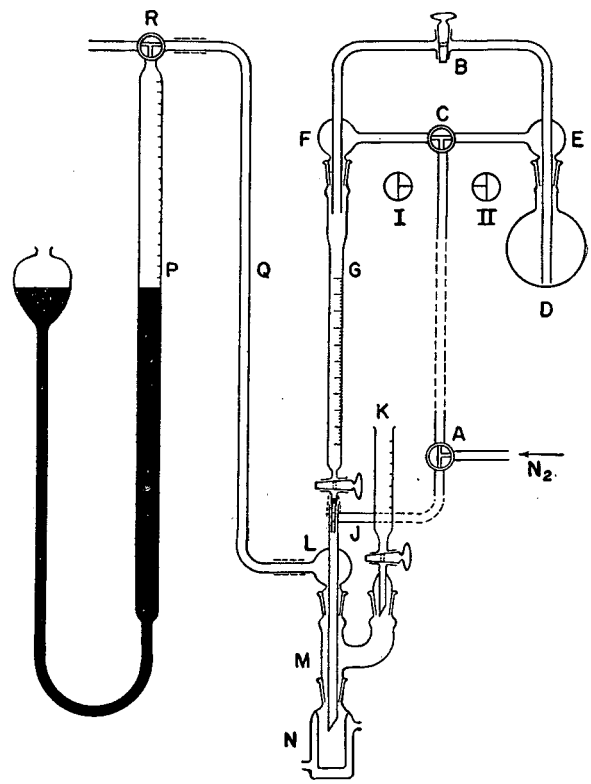

Figure 1. Diagram of Apparatus

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Table I. Action of Methylmagnesium Iodide on Organic Compounds

Compound	Moles of Active Hydrogen/Mole	Moles of Reagent Adding/Mole
Di-*n*-butylamine	0.99 ± 0.02	[a]
Methylaniline	0.96 ± 0.01	[a]
Piperidine	0.99 ± 0.01	[a]
Myristyl alcohol	0.97 ± 0.03	[a]
n-Butyl alcohol	1.01 ± 0.03	[a]
γ-Chlorobutyronitrile	0.01 ± 0.01	0.97 ± 0.02
n-Hexaldehyde	0.00 ± 0.01	0.99 ± 0.02
Methyl *n*-butyl ketone	0.02 ± 0.02	0.98 ± 0.03
Methyl isobutyl ketone	0.03 ± 0.02	1.02 ± 0.04
Methyl *n*-amyl ketone	0.01 ± 0.02	1.05 ± 0.03
Acetophenone oxime	1.02 ± 0.08	0.88 ± 0.08
Phenyl tolyl ketimine	0.98 ± 0.09	1.02 ± 0.05

[a] Not measured.

in the system, and a measured volume, about 1 ml., of the reagent is run into flask *N*. (The contents of this flask are usually mixed by shaking, but stirring can be readily accomplished by the use of a small magnetic stirrer, externally operated. A device of this type is sold by Arthur H. Thomas Co.) After gas evolution has ceased, the level of the mercury is again read and the difference between the two readings is equal to the blank value on the solvent. At this point, a measured volume of water, about 3 ml., is added from buret *K*, and when gas evolution has again ceased, a third reading is taken. [Although the use of water is suggested as the means of decomposing the Grignard reagent, any suitable liquid of lower vapor pressure may be used in this apparatus—for example, aniline, as recommended by Assaf and Gladding (*1*).] The total amount of gas evolved divided by the volume of Grignard solution used is equal to the "methane equivalent" of 1 ml. of the reagent.

Active Hydrogen Determinations and Measurement of Addition Reactions. Active hydrogen analyses are run in the manner described above, except that a solution of the sample in 5 ml. of butyl ether is used instead of the solvent alone. Preceding each run, flask *N*, connecting tube *M*, and buret *K* are removed and washed with 20% hydrochloric acid, water, alcohol, ether, and absolute ether in that order.

Reactions involving consumption of the Grignard reagent without the formation of methane, as in the case of many addition reactions, are measured by adding a known volume of the reagent to a solution of the sample, allowing a definite time for the completion of the reaction, and decomposing the excess reagent by the addition of a measured volume of water. The difference between the amount of methane produced and the amount of methane calculated to be formed from the volume of the Grignard solution is a measure of the amount of the reagent consumed in the reaction.

ACCURACY OF RESULTS

Table I includes some results obtained with the apparatus on various types of organic compounds. The reproducibility of the results is shown by the indicated precision. In the case of methylaniline, for example, values of 0.96, 0.94, 0.99, and 0.96 were obtained.

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RECEIVED July 2, 1947.

Semimicrodetermination of Arsenic in the Presence of Antimony, Bismuth, Tin, and Lead

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THE material given in this paper is an extension of the method described by Sloviter, McNabb, and Wagner (*2*) for the semimicrodetermination of arsenic in organic compounds. The arsenic is precipitated as the element by action of hypophosphorous acid and determined iodometrically with the aid of Koppeschaar's bromide-bromate solution. This method is applicable to the determination of arsenic in the presence of antimony, bismuth, tin, and lead when present as the chlorides.

Table I. Determination of Arsenic

Arsenic Taken *Mg.*	Metals Present *Mg.*	Arsenic Found *Mg.*	Error %
19.16	None	19.18	+0.1
19.16	None	19.16	0.0
19.16	None	19.22	+0.3
19.16	None	19.18	+0.1
19.16	None	19.26	+0.5
19.16	None	19.15	−0.0
19.16	None	19.07	−0.4
19.16	None	19.10	−0.3
19.16	100 Antimony	19.13	−0.1
19.16	100 Antimony	19.12	−0.2
19.16	100 Bismuth	19.01	−0.7
19.16	100 Bismuth	19.01	−0.7
19.16	100 Tin	19.13	−0.1
19.16	100 Tin	19.15	0.0
19.16	100 Tin	19.24	+0.4
19.16	150 Tin	19.09	−0.3
19.16	100 Lead	19.12	−0.2
19.16	100 Lead	19.07	−0.5
19.16	100 Antimony, bismuth	19.08	−0.4
19.16	100 Antimony, bismuth	19.10	−0.3
19.16	200 Antimony, bismuth, and tin	19.17	+0.1
19.16		19.17	+0.1
19.16	100 Antimony, bismuth, tin, and	19.19	+0.2
19.16	lead	19.20	+0.2

Results are given showing the percentage error obtained when arsenic is determined in the presence of these foreign ions.

PROCEDURE

An aliquot portion of sodium arsenite solution containing approximately 20 mg. of arsenic was transferred to the flask of an all-glass decomposition apparatus with reflux tube, such as that described for use in the determination of arsenic or mercury in organic compounds (*1*, *2*). Measured volumes of solutions of antimony chloride, bismuth chloride, stannous chloride, and lead acetate were added to the arsenic solution. To the solution in the flask were added and dissolved rapidly 3 grams of sodium hypophosphite ($NaH_2PO_2H_2O$), and then sufficient concentrated hydrochloric acid was added to increase the acid concentration to at least 6 *N*. The condenser was attached, the flask heated with a small flame, and the analysis completed as described (*2*).

The results obtained for the determination of arsenic alone gave an average error of 0.2% (Table I). Arsenic with antimony present gave an average error of 0.15%; with bismuth 0.7%; with tin 0.2%; and with lead 0.3%. Arsenic with antimony and bismuth present gave an average error of 0.3%; with antimony, bismuth, and tin 0.1%; and with antimony, bismuth, tin, and lead 0.2%.

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RECEIVED December 20, 1947. Presented before the Meeting-in-Miniature of the Philadelphia Section of the AMERICAN CHEMICAL SOCIETY, January 22, 1948.

Semimicro Molecular Still

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IN THE course of studies dealing with the effects of radioactivity on organic compounds (*2*, *4*, *7*, *8*) the quantity of conversion product available for identification has generally been limited to less than 100 mg. Within the past two years, however, advances in the design of cyclotron bombardment chambers (*3*) have made larger yields possible, so that at present up to 5 grams may be obtained for identification by chemical and physical means.

During a recent study of the effects of deuterons on cyclohexanecarboxylic acid (*2*), 4 ml. of a viscous, yellow, highly fluorescent, nonsaponifiable oil were obtained as one of the major conversion products. Attempts to vacuum-distill 1 ml. of this material under a pressure of 1 mm. of mercury in a jacketed, helical fractionating column were unsuccessful, as the liquid decomposed and darkened on continued heating. It seemed advisable, therefore, to turn to short-path distillation as a means of separating the liquid into its components.

Among the small scale molecular stills reported in the literature, those designed by Matchett and Levine (*5*) and Wollner, Matchett, and Levine (*9*) require continuous heating of charge. On the other hand, the falling film still designed by Quackenbush and Steenbock (*6*) operates on a relatively large scale, as it depends upon a pump for recycling the charge.

In order to eliminate thermal decomposition due to prolonged heating, a vertical still (Figure 1), both ends of which were nearly identical, was designed for use with falling film flash distillation. Recycling of charge is carried out by rotation of the still about the lead to the vacuum system. Distilland placed in one end is allowed to fall through the still into the other end. After removal of distillate, the still is turned through 180° and the process is then repeated. In this way it is possible to separate less than 5 ml. of liquid into a requisite number of fractions.

CONSTRUCTION

The distillation surface, *J* (Figure 2), is a 28 × 180 mm. section of Pyrex tubing, on the inner surface of which a spiral of 1-mm. platinum wire is closely wound so as to give 10 turns at a pitch of approximately 15°. The ends of the still are identical, with the exception of joint *O*.

Condensation takes place on 3-mm. Pyrex water condenser *G*, concentric with the distilling surface and extending through both ends of the still. A small cup, *Q* (Figure 3), on each end of the condenser serves to deflect condensate out of the still proper and into collecting bulb *R* or *D*.

Nondistilled liquid is collected in trough *L*, from which it can be concentrated into bulb *P* or *A* for redistillation at a higher temperature.

A Nichrome heating element, *H*, current to which is regulated by a variable transformer, surrounds the distilling surface, *J*, and enables operation at different temperatures. Temperature measurement is by means of a copper-constantan thermocouple, one junction of which is inserted between the still and heating jacket.

Pressure is measured by a McLeod gage connected to the apparatus by 8-mm. tube *F*. The entire system is evacuated through 28-mm. tube *K*, a dry ice–acetone trap, mercury diffusion pump, and Hyvac fore-pump.

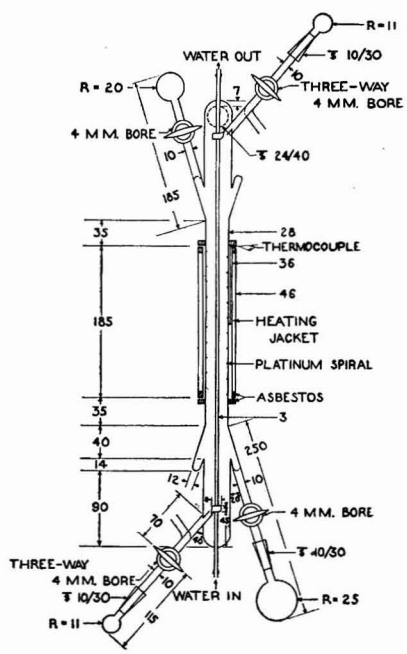

Figure 1. Semimicro Molecular Still

All dimensions in mm.

OPERATION

Figure 2 shows the still in position for the introduction of sample into bulb *P*. With stopcock *N* open and the entire still under a vacuum of approximately 1 micron, the sample can be readily degassed by immersing bulb *P* in a beaker of water at 80° to 100° C. for several minutes. After stopcock *N* is closed, the still is rotated through 180° about joint *C*, so that it assumes the position shown in Figure 4. By carefully adjusting stopcock *N*, the flow of material to be distilled can be controlled so that the liquid will follow the path of the platinum spiral without running over its edge. Flow over or under the edge of the platinum wire is to be avoided, because the liquid tends to stream rather than to spread to a thin film.

After the liquid has drained from bulb *P* (Figure 4) approximately 0.5 hour is allowed for it to drain from the distilling surface into the collection bulbs, *A* and *D*. By turning stopcock *E* through 180° the distillate is brought to atmospheric pressure and can be removed. A clean distillate receiver is pre-evacuated by the forepump through stopcock *E* and its arm, *M*. On the other end of the still, arm *I* is used for this same purpose.

Stopcock *B* is closed and the still is again rotated through 180° about joint *C*. The distillation is now repeated with the temperature of the distilling surface about 10° C. higher than it was during the previous cycle.

EXPERIMENTAL

The still was used in an effort to separate the components of the nonsaponifiable oil obtained from the bombardment of cyclohexanecarboxylic acid. A 4-gram charge of the oil was subjected to molecular distillation by the procedure described above, and

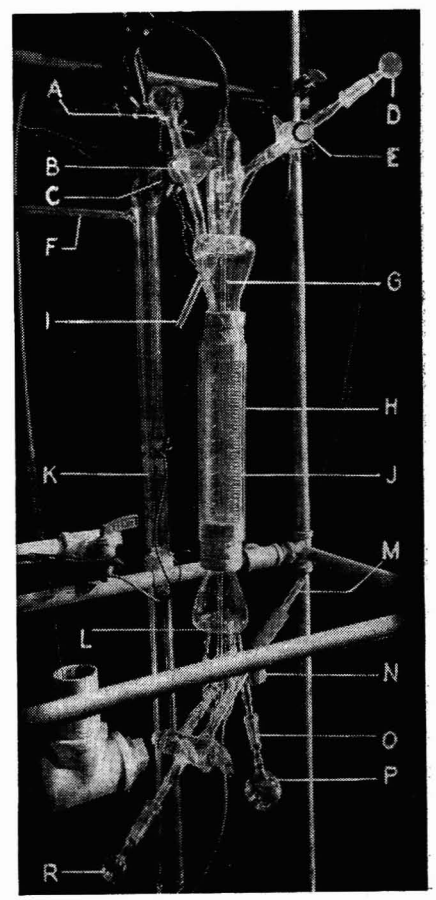

Figure 2. Semimicro Molecular Still in Position for Sample Introduction and Degassing

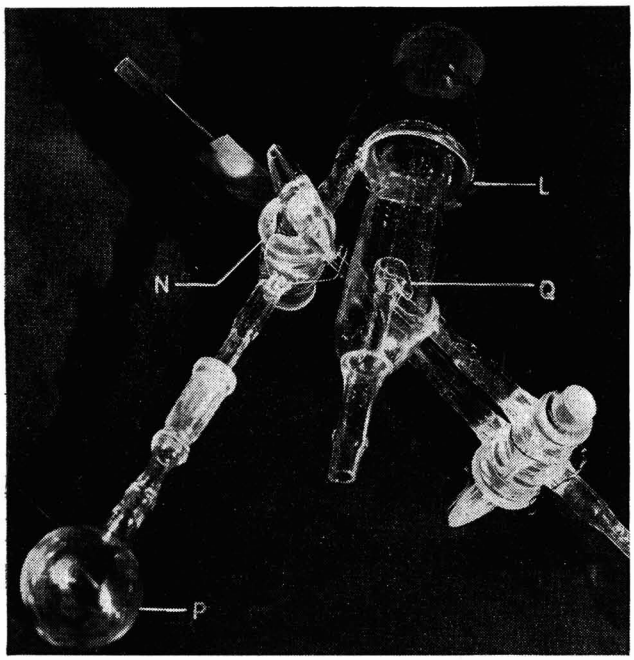

Figure 3. Close-Up of Distillate Removal Cup

the fractions listed in Table I were collected. The first fraction recorded was low boiling and escaped from the still into the dry ice–acetone trap, from which it was recovered. As shown in Table I, the physical properties of fractions 2 and 3 indicated that the major portion of the oil was probably dicyclohexyl ketone.

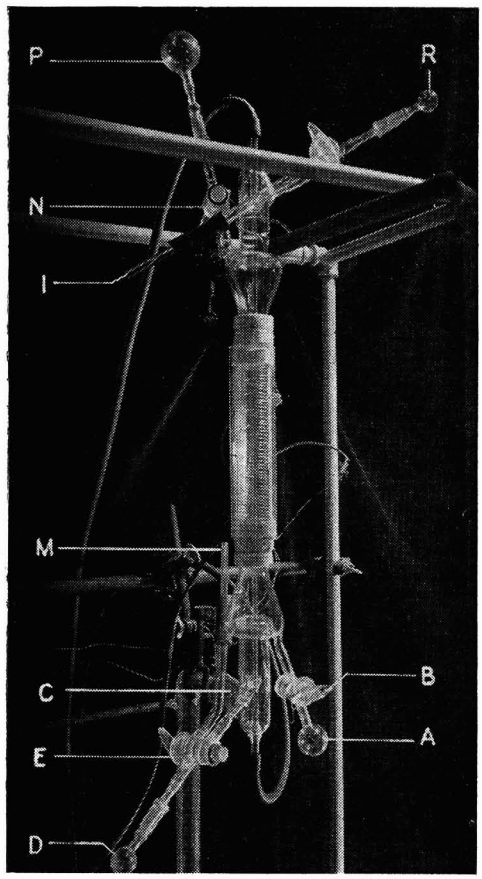

Figure 4. Semimicro Molecular Still in Standing Position

Table I. Physical Properties of Fractions Obtained by Molecular Distillation of Oil from Deuteron Bombardment of Cyclohexanecarboxylic Acid

	n_D^{20}	d_4^{20}	Distillation Temperature, ° C.
Original oil	1.4985	0.980	
Fraction 1	1.4771	0.935	(25)
Fraction 2	1.4822	0.943	25
Fraction 3	1.4875	0.987	50
Residue	1.5078	...	.....
Dicyclohexyl ketone (*1*)	1.4851/14°	0.986	.....

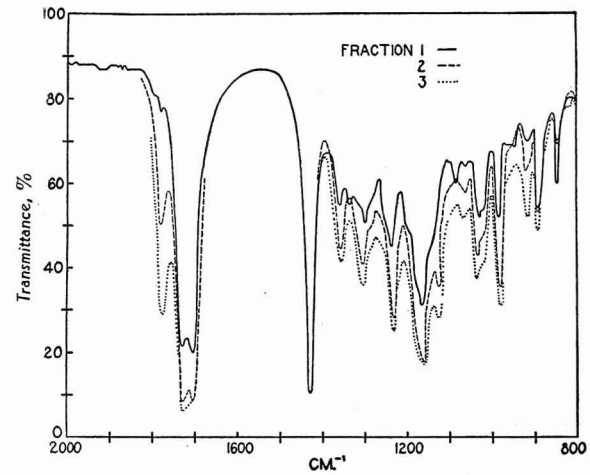

Figure 5. Infrared Spectra of Fractions Obtained by Molecular Distillation of Nonsaponifiable Oil from Deuteron Bombardment of Cyclohexanecarboxylic Acid

To obtain further information regarding the distilled material each fraction was submitted for infrared analysis, using a Perkin-Elmer instrument. The spectra thus obtained (Figure 5) showed strong absorption in the carbonyl region (1700 to 1750 cm.$^{-1}$) and offered further evidence for dicyclohexyl ketone.

Because all the spectra were obtained under the same conditions using a fixed cell, variations of the fractions in the regions of 1080, 1140, 1350, and 1750 cm.$^{-1}$ indicated that this still is capable of separating and concentrating the components of a heavy oil.

CONCLUSIONS

A semimicro molecular still has been designed which employs the falling film principle to eliminate continuous heating of the sample, and makes possible recycling of the sample without use of a pump. By means of an experimental run it has been demonstrated that this apparatus can be used for the fractionation of small quantities of heat-sensitive oils at low temperatures.

ACKNOWLEDGMENT

Sincere appreciation is extended to Clark Goodman of the Massachusetts Institute of Technology, physics department, for his suggestions regarding this work. Appreciation is also extended to Virginia L. Burton for assistance with the experimental work, and to Earle C. Farmer for the infrared curves. The assistance of Walter Ennis, Massachusetts Institute of Technology, physics department glass blower, is acknowledged.

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RECEIVED October 6, 1947. Contribution from American Petroleum Institute Research Project 43C located at the Massachusetts Institute of Technology; W. L. Whitehead, director; Clark Goodman, physical director.

Simple Electronic Reflux Ratio Timer

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IN THE course of the past few years, the increasing popularity of magnetically operated heads for distilling columns (Oldershaw, Du Pont, Piros-Glover) has made a flexible, low-cost timer desirable. Earlier controls based on single synchronous clock motors were reliable and cheap, but suffered from the restrictions imposed by simple proportioning of a fixed total period. More elaborate double synchronous clock motors eliminated this difficulty, but required considerable maintenance, usually had a fixed "on" period, and were relatively expensive.

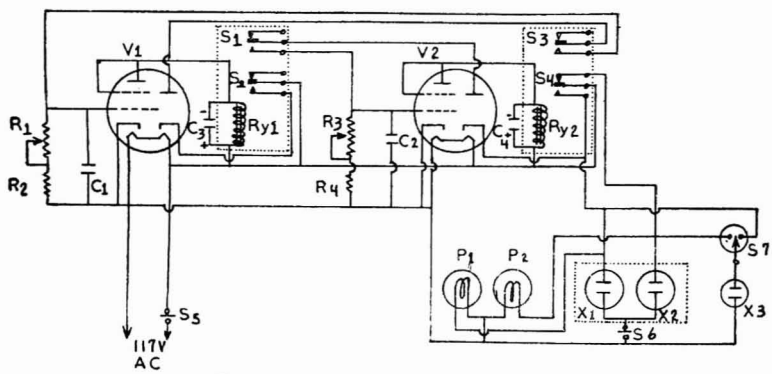

Figure 1. Schematic Diagram

R_1. 3-megohm carbon potentiometer
R_2. 250,000-ohm, 0.5-watt resistor
R_3. 1-megohm carbon potentiometer
R_4. 50,000-ohm, 0.5-watt resistor
C_1. 4-mfd. paper condenser, 400-volt
C_2. 2 mfd. paper condenser, 400-volt
C_3, C_4. 8-mfd. electrolytic condensers, 250-volt
Ry_1 (S_1S_2). Double-pole double-throw relay, 1000- to 4000-ohm coil
Ry_2 (S_3S_4). Double-pole double-throw relay, 1000- to 4000-ohm coil
V_1, V_2. 117 L7/M7-GT tubes
S_5, S_6. Single-pole single-throw toggle switches
S_7. Single-pole double-throw toggle switch
X_1X_2. Duplex receptacle (see text)
X_3. Single receptacle
P_1, P_2. 110-volt S_5 pilot lights

Recently Thacker and Walker (*4*) described an electronic timer which met most of the requirements, but had the disadvantage of rather specialized, difficultly obtainable parts. The two-tube electronic timer described below is reliable and flexible and is easily constructed at very low cost (under $20).

The circuit is based on the well-known condenser discharge principle, which has been used, in various forms, for pump control (*1*, *3*), spectrographic exposure control (*2*), and time delay relays. It consists of two interlocked timing units, one for "off" or reflux timing, the other for "on" or delivery timing. Both periods may be varied over a wide range by selection of proper resistance and capacitance values. The constants given in Figure 1 serve only as a guide, and may be changed to suit the application of the device.

The operation of the timer may be easily understood by considering separately the action of each tube. When switch S_5 is closed, tube V_1 warms up and the beam power section acts as a self-rectifying amplifier, drawing current through relay Ry_1 and closing its contacts, S_1 and S_2. The diode rectifier section of V_1 then applies a negative bias to the control grid of V_1 beam tetrode through contacts S_2 of Ry_1 and S_3 of Ry_2 (the latter has closed by this time, analogous to Ry_1). As the negative voltage, stored by C_1, on the control grid of V_1 biases the beam power section to cutoff, Ry_1 drops out and remains open until the charge on C_1 has leaked off through R_1R_2. The time interval during which Ry_1 is energized is controlled by the setting of R_1 and, with the constants given, may be varied over a period of 4 to 60 seconds. When the negative charge has leaked off the control grid of V_1, Ry_1 pulls in once more and the cycle is repeated. V_1 thus delivers a repetitive pulse (through contacts S_1 of Ry_1) at a constant, controllable rate.

The action of V_2, triggered by the pulse from V_1, is strictly analogous, except that the output circuit does not pulse. The constants given in the schematic diagram result in a delivery period of 1 to 5 seconds, variable by R_3. V_1 and V_2 are interlocked through contacts S_3 of Ry_2 to prevent V_1 from pulsing unless V_2 has completed its timing cycle. The output of V_2 (through Ry_2) is delivered to X_1X_2, an ordinary duplex receptacle with one of the connecting straps divided into two, which enables the timer to operate directly a reflux dividing head activated by two alternately energized magnets. The more common type of single solenoid head may be activated from receptacle X_2.

The single receptacle, X_3, which may be connected at will to either timing interval by the single-pole double-throw switch, S_7, serves the purpose of initial calibration, or accurate resetting of controls R_1 and R_3 when an electric stop clock is connected to it. Switch S_6 may be opened to de-energize the output circuit without shutting off the timer. Pilot lights P_1 and P_2 indicate which of the output circuits is connected, and may be used for stopwatch timing in calibrating or checking the device.

The timer, as described above, has been in use in the Research Laboratories of Shawinigan Chemicals for the past year, much of the time on 24-hour, 5.5-day-week service. No maintenance or tube replacement has been required during this period. Constancy of timing over 24-hour periods has been better than 1%.

ACKNOWLEDGMENTS

The author wishes to acknowledge gratefully the encouragement of K. G. Blaikie, director of the Research Laboratories, Shawinigan Chemicals, Ltd., and to thank Shawinigan Chemicals, Ltd., for permission to publish this work.

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Fluorocarbon Vapor Balance

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FOR the determination of molecular weights of fluorocarbons boiling below 100° C., the gas density balance previously described (*1*) is very satisfactory. For substances boiling 100° C. or above, the vapor pressures at room temperature are too low for the use of this instrument. The device shown in Figure 1 was designed and found to function well (Table I).

It consists essentially of a glass boiler so arranged that a static body of vapor at constant temperature can be maintained in a container. In this vapor a glass bob is suspended by a fine wire, which is held from above on one side of an analytical balance. Weighing the bob in the vapor and determining the temperature with a thermocouple provide the necessary data for the calculation of molecular weight. The balance rests on a metal plate, the horizontal motion of which can be slightly changed by means of setscrews. This is for the purpose of centering the bob.

In operation the instrument is mounted securely on a frame. The position of the balance is adjusted by the setscrews until the bob floats freely in the chamber. The weight of the bob in air is determined, the sample is then introduced through the left condenser, and the temperatures of the various sections of the apparatus are adjusted by the amount of current through the heaters. The left and bottom (*R* and *S*) heaters provide a temperature approximating the boiling point of the liquid. The bob chamber, *J*, is adjusted to about 50° C. higher and the wire heater, *B*, about 15° C. higher than the boiling point of the liquid. The pot heater, *V*, at first provides reflux in both condensers. In a short time air is expelled from the bob chamber, and the current to the pot heater is reduced until the reflux level is about 25 mm. above the bottom of the left condenser. As soon as temperatures become constant, the bob is weighed. Weighings are constant for about 15 minutes, after which the diffusion of air into the vapor causes a slight decrease in the measurement.

The instrument functions on less than 1.5 ml. of liquid and the precision of the measurements is within ±1%.

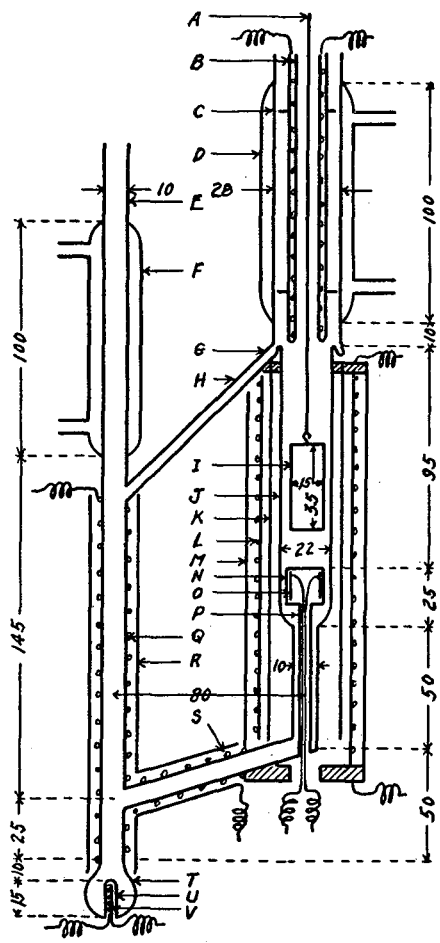

Figure 1. Fluorocarbon Vapor Balance

Table I. Molecular Weights of Fluorocarbons

Sample No.	Boiling Range ° C.	Temp. Chamber ° C.	Pressure *Mm. Hg*	Wt., Bulb in Vapor *Grams*	Apparent Molecular Weight
1	101	148	736	4.9670	442
		148	736	4.9672	442
		184	735	4.9741	442
		187	735	4.9748	441
2	99	180	735	4.9802	403
		185	735	4.9810	404
		185	735	4.9810	404
3	178–188	240	735	4.9615	577
		244	735	4.9618	578
		245	735	4.9619	579
4	220–238	284	740	4.9515	691
		285	740	4.9515	693
5	280–300	355	742	4.9418	851
		355	742	4.9420	850
		355	742	4.9424	848

Samples are fluorocarbons of different boiling ranges. 1 is reasonably pure C_8F_{18} (molecular weight 438).
Duplicate determinations at same and different temperatures for chamber show reproducibility.
Bulb volume, 6.78 cc. Bulb weight (in vacuum), 5.0514 grams.

ACKNOWLEDGMENT

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The authors wish to express their thanks for the assistance of C. W. Brouse of the school shops and F. J. Malloy, the school glass blower.

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RECEIVED November 1, 1947.

All figures in millimeters

A. Wire support from analytical balance above (not shown) to glass bob, No. 40 nickel wire
B. Heater to keep wire free from condensate Nichrome wire winding between two glass cylinders sealed at bottom. Free space between glass walls filled with Alundum cement
C. Prongs on *B* to center. On lower end (not shown) additional prongs give vertical support by resting on *G*
D. Glass condenser jacket, 38 mm. o.d.
E. Glass reflux tube
F. Glass condenser jacket, 22 mm. o.d.
G. Circular well to catch reflux from condenser
H. Return tube to drain liquid from *G*, 6 mm. o.d.
I. Glass bob, full of air but sealed
J. Glass envelope of bob chamber, 22 mm. o.d.
K. Cylindrical copper jacket to maintain uniform temperature in bob chamber
L. Nichrome winding on cylindrical glass envelope, for heating bob chamber
M. Glass external jacket
N. Thermometer chamber
O. Thermometer. A ring made of short copper tubing to which Chromel-Alumel thermocouples are spot-welded. Temperatures determined by potentiometer readings
P. Glass thermocouple wire tube
Q. Nichrome winding to heat connecting tubes
R. Asbestos insulation covering for connecting tubes
S. Winding *Q* and insulation *R* extend around lower connecting tube
T. Glass boiling pot
U. Glass re-entry heater tube
V. Nichrome heater for pot

Elimination of Nitrite Interference in Iodometric Sugar Determinations

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IT IS frequently desired to determine reducing sugars by iodometric methods (ferricyanide or copper reduction followed by iodometry) in bacteriological culture media containing nitrites. However, erroneous sugar values are obtained when such a medium is assayed by any of these methods, as for instance, the Somogyi procedure (*2*). The cause of the error lies in the fact that potassium iodide, in an acidified sodium nitrite solution, produces free iodine. It is obvious, therefore, that nitrites must be eliminated before one proceeds with the sugar determination.

In the Groak (*1*) iodine microdetermination excess sodium nitrite is eliminated by addition of urea to the hot acid solution.

$$H_2NCONH_2 + 2HONO \longrightarrow CO_2 + 2N_2 + 3H_2O$$

Adaptation of this procedure for the elimination of nitrite interference prior to iodometric sugar determinations was therefore investigated.

Ten milliliters of 1.0% sodium nitrite solution and 10 ml. of 10.0% urea were mixed in a 100-ml. volumetric flask. Five milliliters of 1 *N* sulfuric acid were added. The flask was shaken occasionally and after 30 minutes 2 drops of phenolphathalein were added. The solution was neutralized with sodium hydroxide and made up to volume with distilled water. Assay of a 5-ml. aliquot resulted in a titration of 24.50 ml. of thiosulfate, equal to the blank titration. Thus in the absence of sugar, nitrite interference had been eliminated completely.

The efficacy of this procedure was tested with different sugars.

Ten milliliters of a glucose solution were pipetted into each of three 100-ml. volumetric flasks. The solution in one flask was diluted to volume and assayed in the regular manner as a control. Ten milliliters of 1.0% sodium nitrite solution were added to each of the other two flasks. One of these was diluted to the mark and assayed to show the extent of nitrite interference. The remaining flask was treated with an excess of urea and sulfuric acid as indicated above, to show removal of nitrite interference. Similar experiments were performed with maltose and sucrose solutions.

Table I. Elimination of Nitrite Interference in Iodometric Sugar Determinations

10-Ml. Aliquot in 100-Ml. Volumetric Flask	Treatment	0.005 *N* Thiosulfate Titration	Titration Difference[a]
		Ml.	*Ml.*
0.3% glucose	None (control)	14.20	10.30
	Nitrite	47.10	Negative
	Nitrite + urea + H_2SO_4	14.20	10.30
0.53% maltose	None (control)	13.60	10.90
	Nitrite	45.00	Negative
	Nitrite + urea + H_2SO_4	13.60	10.90
1.0% sucrose	None (control)	24.45	0.05
	Nitrite	45.50	Negative
	Nitrite + urea + H_2SO_4	24.25	0.25

[a] Titration resulting from presence of sugar minus reagent blank. Reagent blank = 24.50 ml.

Results given in Table I show that interference by nitrite, in the presence of sugars, was completely eliminated. Sucrose was apparently hydrolyzed to a negligible extent by the mild treatment with sulfuric acid while maltose was not affected.

Not only are nitrites commonly added as such to culture media, but they are sometimes formed by the action of microorganisms, as from the oxidation of ammonia or the reduction of nitrates. Regardless of the source of nitrite interference, this interference can be eliminated effectively as described above.

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Removal of Oxygen from Gas Streams

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THE most popular agents for removing oxygen from gases are hot finely divided copper and a train of chromous sulfate solutions. Although either absorbs oxygen substantially completely, both have disadvantages that render their use inconvenient.

Not only is a copper heater somewhat tedious to construct and place in operation, but much time is required to attain operating temperature: these are serious drawbacks when the apparatus is to be used only infrequently. Chromous sulfate solutions, generally prepared by the reaction of metallic zinc and acidified chromic sulfate, must be allowed to stand for many hours before they are ready for use. Further, because the hydrous chromic oxide formed on aging is dissolved and reduced only slowly on addition of acid, the maintenance of these solutions in operating condition is a source of constant annoyance.

The authors have found that vanadous sulfate solutions present none of these difficulties. A solution initially 0.1 *M* in vanadyl sulfate and containing some free sulfuric acid is ready for use within a minute or two after amalgamated zinc is added, and regeneration by addition of sulfuric acid (conveniently through a small separatory funnel whose stem projects through the stopper of the wash bottle) proceeds so rapidly that the solutions are again ready for use almost instantly.

Unlike alkaline suspensions of hydrous chromic oxide, alkaline suspensions of hydrous vanadic oxide are highly effective in oxygen removal, being oxidized to vanadite, which itself strongly absorbs oxygen and is converted to vanadate. Therefore the absorptive capacity of a vanadous sulfate solution is much greater than that of an equiconcentrated chromous sulfate solution, and regeneration is necessary much less frequently.

The authors have used a train of three wash bottles, the first two of which were initially charged with 100 grams of lightly amalgamated zinc and 100 ml. of 0.1 *M* vanadyl sulfate, and the third contains 100 ml. of water to ensure absence of vanadium from the emergent gas stream. This assembly has now been in operation for over 9 months, and has been regenerated only twice. Its performance in nearly continuous service has been eminently satisfactory.

Tank nitrogen passed through this train was used to remove dissolved air from 50 ml. of air-saturated 0.1 *N* potassium chloride, 0.005 *M* sodium hydroxide, and 0.01% gelatin contained in a polarographic H-cell (*2*), and the oxygen diffusion current at

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$E_{d.e.}$ = −1.5 volt *vs.* the saturated calomel electrode was measured, after 15 minutes' bubbling, with a polarographic assembly in which a Type K potentiometer was used to measure the potential drop across a 10,000-ohm standard resistance in series with the dropping electrode. As shown by the constancy of the measured currents, this length of time sufficed for the establishment of equilibrium in every case. Residual currents were then secured after addition of 5 ml. of 0.4 *M* sodium sulfite to remove oxygen completely (*1*).

With unpurified tank nitrogen, the equilibrium oxygen diffusion current was 0.0027 microampere; when the nitrogen was passed through the vanadous sulfate train, this current was only 0.0007 microampere, which is equal to zero within the probable uncertainty of the polarographic measurements. When nitrogen intentionally contaminated with air from the laboratory compressor was used, passage through the vanadous sulfate train reduced the oxygen diffusion current at equilibrium from 0.051 to 0.0003 microampere. These data prove the efficiency of the proposed method for oxygen removal.

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RECEIVED March 12, 1948.

SCIENTIFIC COMMUNICATIONS

Equilibrium and Kinetic Studies on the Formation and Dissociation of Ferroin and Ferriin

THE reaction of *o*-phenanthroline with ferrous iron to form ferroin is of great analytical importance in connection with the colorimetric determination of iron. We have found that phenanthroline behaves strictly as a monoacidic base in aqueous solutions and that the acid dissociation constant of the phenanthrolium ion is 1.1×10^{-5} at 25.0° C. The equilibrium between ferrous iron and phenanthroline in acid solution was found to be represented by either of the following expressions, in which Ph and PhH^+ denote phenanthroline and phenanthrolium ion, respectively.

$$Fe^{++} + 3Ph \rightleftharpoons FePh_3^{++} \quad (1)$$

$$Fe^{++} + 3PhH^+ \rightleftharpoons FePh_3^{++} + 3H^+ \quad (2)$$

The equilibrium constants were found to be:

$$K'_{\text{ferroin}} = \frac{(Fe^{++})(Ph)^3}{(FePh_3^{++})} = 5 \times 10^{-22} \text{ at } 25° \quad (3)$$

$$K''_{\text{ferroin}} = \frac{(Fe^{++})(PhH^+)^3}{(FePh_3^{++})(H^+)^3} = 4 \times 10^{-7} \text{ at } 25° \quad (4)$$

It is evident from Equation 4 that the quantitative conversion of ferrous iron to ferroin in acid solutions is dependent on the ratio of excess phenanthroline to acid. In order that the reaction be 99% complete, the ratio of excess (with reference to ferrous iron) phenanthrolium to hydrogen ions must be 0.035 or greater in the equilibrium mixture. A consideration of the effect of excess phenanthroline is also of importance in the colorimetric determination of ferrous iron in the presence of other metal ions which form complexes with phenanthroline. This is being studied.

Assuming that the dissociation of ferriin is represented by an expression similar to Equation 1:

$$K'_{\text{ferriin}} = \frac{(Fe^{+++})(Ph)^3}{(FePh_3^{+++})} = 8 \times 10^{-15} \text{ (in 0.05 } M \text{ } H_2SO_4 \text{ at 25°)} \quad (5)$$

The rates of formation and of dissociation of ferroin are of importance in connection with the colorimetric determination of iron (and of phenanthroline), while the rates of dissociation of ferroin and ferriin are of practical consequence in connection with the use of ferroin as an oxidation-reduction indicator. The dissociation of ferroin is a first-order reaction, the rate of which is independent of the acidity of the solution.

$$v = k_{\text{diss. ferroin}} (FePh_3^{++}); \; k_{\text{diss. ferroin}} = 0.0045 \text{ min.}^{-1} \text{ at } 25° \quad (6)$$

Consequently, the half-life period of ferroin in acid solutions is about 2.5 hours at 25° C. The decomposition of ferriin is a first-order reaction with a rate constant of the same order of magnitude as that of ferroin.

The rate of formation of ferroin is given by the expression:

$$v = k_{\text{form. ferroin}} (Fe^{++})(Ph)^3; \; k_{\text{form. ferroin}} = 1.3 \times 10^{19} \text{ min.}^{-1} \text{ at } 25° \quad (7)$$

In acid solutions the rate can also be represented by:

$$v = k'_{\text{form. ferroin}} \frac{(Fe^{++})(PhH^+)^3}{(H^+)^3}; \; k'_{\text{form. ferroin}} = 2 \times 10^4 \text{ min.}^{-1} \text{ at } 25° \quad (8)$$

I. M. KOLTHOFF
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D. L. LEUSSING

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Minneapolis, Minn.

Replica Studies of Dyed Nylon—Correction

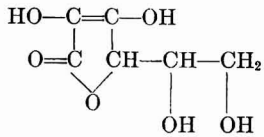
IN THE article on Replica Studies of Dyed Nylon [ANAL. CHEM., 20, 861 (1948)], the following errors occurred. The next to the last sentence in the abstract (page 861) should read: "The ability to fingerprint a dyestuff on a fiber using only a few milligrams of cloth and a few micrograms of dyestuff makes this type of specimen preparation very interesting." The fourth sentence in the fourth paragraph of the section entitled "Electron Diffraction" (page 870) should read: "A few milligrams of each of the three nylon cloths dyed with a few micrograms of dyestuff adequately served as a sample for the identification of the three dyes under discussion." In the section entitled "Conclusions" the first sentence in the fourth paragraph should read: "These two observations can be used to account for the three modifications in the qualities of dyeings described above." The second sentence in the last paragraph should read: "The ability to identify a dyestuff on a fiber, utilizing a few micrograms of dyestuff on a few milligrams of cloth, precludes the usual rather tedious procedure of extracting relatively large amounts of the dyestuff which might then be identified by other chemical or physical analyses."

F. A. HAMM
J. J. COMER

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

11. Ascorbic Acid



Ascorbic Acid

Ascorbic acid, commonly called vitamin C and less commonly named hexuronic acid, cevitamic acid, or antiscorbutic vitamin, crystallizes from water. The greater part of the microscopical data was obtained by microrecrystallization on a microscope slide, while the lattice parameters were determined on large single crystals obtained by macrorecrystallization from water. Good crystals similar to those from water can be obtained from acetone or methanol.

There was no evidence of polymorphism of ascorbic acid under the conditions of these experiments.

CRYSTAL MORPHOLOGY (checked by W. C. McCrone).

Crystal System. Monoclinic.

Form and Habit. Plates and tablets lying on the orthopinacoid, 100. Usually elongated slightly parallel to *c;* shows the forms: orthopinacoid {100}, clinopinacoid {010}, basal pinacoid {001}, prism {210}, positive orthodome {101}, and negative orthodome {$\bar{2}$01}.

Axial Ratio. $a:b:c = 2.703:1:1.008$.

Interfacial Angles (Polar). 011∧0$\bar{1}$1, 90° 24′; 210∧2$\bar{1}$0, 107° 0′; 101∧100, 59°; $\bar{2}$01∧$\bar{1}$00, 63°.

Beta Angle. 102° 30′ (102½) (*4*).

Cleavage. 010 (pronounced).

X-RAY DIFFRACTION DATA (checked by J. F. Whitney).

Space Group. C_2^2 (P_1^2) (*2*).

Cell Dimensions. a = 17.19 Å.; b = 6.36 Å.; c = 6.41 Å.; (a = 16.95 Å.; b = 6.32 Å.; c = 6.38 Å.) (*1, 3–5, 7, 8*).

Formula Weight. 176.

Density. 1.696 (buoyancy method); (1.74) (*1, 3–5, 7, 8*).

OPTICAL PROPERTIES (Checked by W. C. McCrone)

Refractive Indices (5893 Å.; 25° C.). α = 1.474 ± 0.002; (1.465) (*6*); (1.476) (*1, 3–5, 7, 8*). β = 1.595 ± 0.002; (1.600) (*6*); (1.594) (*3*). γ = 1.746 ± 0.004; (1.747) (*6*); (1.750) (*1, 3–5, 7, 8*). γ' = 1.694 ± 0.002 (in plane of 100).

Optical Axial Angles. (5893 Å; 25° C.). $2V$ = 89° ± 1°.

Dispersion. Moderate horizontal $r>v$ at low wave length with a change to moderate crossed dispersion $v>r$ and change of sign in the far red.

Optic Axial Plane. ⊥010; $\gamma \wedge c$ = 41° ± 1° in acute beta.

Sign of Double Refraction. Positive except in far red, negative.

Extinction: $\gamma \wedge c$ = 41° ± 1° in acute beta.

Molecular Refraction (R) (5893Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.601 ± 0.004. R(calcd.) = 35.6. R(obsd.) = 35.2.

Optical Rotation. $[\alpha]_{5780}$ = + 24° in water.

FUSION DATA (determined by W. C. McCrone).

1. Ascorbic acid melts at 182° C. with no sublimation and very slight decomposition (if carefully and quickly heated).

2. The melt crystallizes spontaneously on cooling unless extensive decomposition has occurred. The crystal front is made up of coarse angular crystals just below the melting point. As the temperature decreases, the crystal size decreases and the crystal front becomes spherulitic. At the same time, the rate of growth decreases until at room temperature it is almost zero. Gas bubbles are very prominent and provide an important identifying characteristic. Very slow growth at room temperature is characterized by tangential shrinkage cracks and no gas bubbles of the usual type.

3. If carefully reheated, ascorbic acid can be partly remelted without decomposition. The crystals growing then just below the melting point grow large; an off-center optic axis or acute bisectrix interference figure can usually be obtained, $2V$ = 88°, $r>v$, (+).

4. Ascorbic acid is rather insoluble in thymol but can be made to crystallize during a mixed fusion with this second component as characteristic dendrites. The refractive index of ascorbic acid melt is much greater than that of thymol (1.52).

Principal Lines

d	I/I_1	d	I/I_1
8.50	0.43	2.83	0.04
6.30	0.18	2.79	0.25
5.55	0.24	2.74	0.38
5.08	0.38	2.68	0.54
4.46	0.86	2.62	0.19
4.19	0.24	2.57	0.13
3.90	Very weak	2.51	0.16
3.76	0.16	2.43	0.09
3.51	0.34	2.39	0.20
3.30	0.15	2.32	Very weak
3.17	1.00	2.26	Very weak
3.09	Very weak	0.23	0.19
2.93	0.85	2.17	0.09
2.90	0.03	2.14	Very weak
		2.09	Very weak

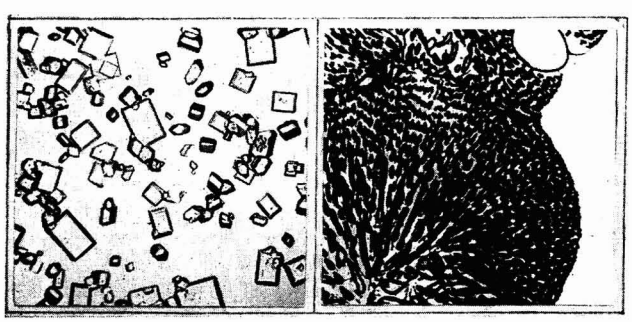


Figure 1. Crystals of Ascorbic Acid

Left. **Obtained from water on microscope slide**
Right. **Growing from melt just above room temperature**

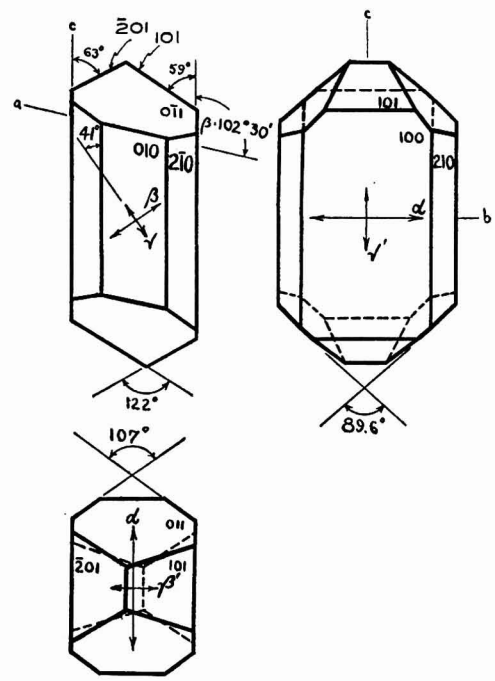


Figure 2. Orthographic Projection of Common Crystal Habit of Ascorbic Acid

The dendrites growing into thymol show the usual 100 view with one refractive index less than that of thymol (alpha, 1.474) and one greater (gamma prime, 1.694).

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(4) Cox and Hirst, *Nature*, **131**, 402 (1933).
(5) Cox, Hirst, and Reynolds, *Ibid.*, **130**, 888 (1932).
(6) Hendricke, *Ibid.*, **133**, 178 (1934).
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BOOK REVIEWS

Dissociation Energies and Spectra of Diatomic Molecules. *A. G. Gaydon.* xi + 239 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, N. Y., 1947. Price $5.

"Spectroscopic Determination of Dissociation Energies of Diatomic Molecules" might be a more accurate title for this rather specialized little book. General characteristics of the spectra of diatomic molecules are briefly outlined, and polite gestures are made in two short chapters toward electron impact, thermal, and thermochemical methods of determining dissociation energies. But the author's announced purposes are to give a full description of that part of molecular spectroscopy which is relevant to the determination of dissociation energies, to collect available data on dissociation energies of diatomic molecules, and finally to discuss in detail several important molecules whose energies have remained ambiguous. These purposes are well fulfilled.

There can be no question about the lasting value of the excellent discussion of spectroscopic principles and criteria for determining dissociation energies or of the useful collection of recommended values and references for dissociation energies of some 250 diatomic molecules. However, the detailed examination of such widely debated cases as nitrogen and carbon monoxide which are included in this book will probably be read carefully only by those who have participated in the debates. The chapter on dissociation energy of carbon monoxide and related molecules furnishes a good example of the hazards of publishing in book form material about which there are considerable uncertainty and controversy. The book is scarcely off the press and already new material is available which will be judged by some to demand a revision of this chapter.

CHAS. H. TOWNES

Spectrographic Analysis of Soils, Plants, and Related Materials. *R. L. Mitchell.* iv + 183 pages. Commonwealth Bureau of Soil Science, Harpenden, England, 1948. Price, 12s. 6d.

This book, designated as Technical Communication No. 44, reviews the literature on spectrographic methods of analysis that are relevant to the investigation of soils, plants, and such related substances as soil parent materials, fertilizers, and animal products. It also presents in some detail the methods of analysis that have been worked out over the past 12 years in the Spectrographic Department of the Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen.

A major portion of the book deals with recognized methods of procedure. In this respect it serves to bring the subject up to date. Both arc and flame emission methods are described in detail. This presentation provides the experienced operator of spectrographic equipment with an accurate and useful review of various methods of procedure. The beginner will find the book a helpful introduction to the work because of its not too technical language.

It is pointed out that although the spectrograph is a powerful analytical tool, overenthusiastic claims at times have been made for it. The technique is most effective when used in conjunction with standard analytical chemical methods. Some types of analysis can be carried out more easily and accurately by chemical methods, others by the spectrograph. It is desirable, therefore, that the analyst appreciate the particular advantages of both techniques.

The chapter on the preparation of soils, plants, and other materials gives such specific directions that the operator should be able to reproduce essentially the author's procedures.

The extensive material the author has brought together should be of great value to the spectrographer as he meets the varied problems of his field. The bibliography of 27 pages appears to cover the literature splendidly. The book should be in the library of everyone engaged in the chemical analysis of agricultural materials by spectrographic means.

ALSTON W. SPECHT

Quantitative Analysis. *Willis C. Pierce* and *Edward L. Haenisch* 3rd ed. xiv + 520 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York, 16, N. Y., 1948. Price, $3.75.

The third edition of this well-known text has been almost completely rewritten and brought up to date. Some of the notable changes include the following: The electrochemical sign convention has been changed; the negative sign is used for oxidizing agents and the positive sign for strong reducing agents. This convention conforms to that used in most physical chemistry texts. Arsenious oxide is recommended as a primary standard for permanganate. The mixed indicator, methyl red–bromocresol green, is recommended for the carbonate titration. The theoretical aspect of precipitation has been expanded into three chapters. An added chapter gives more material on complex equilibria. The chapter on colorimetry has been omitted, the authors' opinion being that this subject should be treated in an advanced course on instrumental analysis. There are numerous additions and some deletions in the laboratory exercises. Questions and problems at the ends of the chapters have been rewritten and nearly doubled in number. Answer sheets are available from the publisher, on order of the instructor.

The chapters in the book involving electrochemical equilibria are especially well written. The material is well organized, clearly presented, and very well explained. In the chapter on electrodeposition, however, under the paragraph on overvoltage, decomposition potential is defined as the potential calculated from the values of half-cell potentials, which therefore give theoretical values for the decomposition potentials. This is not in accordance with the definition given by physical chemists, who generally agree that the decomposition potential is the experimentally determined value for the potential of the reverse process when an external e.m.f. is applied between the electrodes, and therefore includes overvoltage.

This reviewer considers the book as one of the outstanding fundamental texts in analytical chemistry. It is thoroughly modern and clearly written, and incorporates the writers' teaching experience with the textbook material. The authors are to be commended for their efforts in the revision of this excellent text.

WILLIAM J. TOMSICEK

Colorimetric Methods of Analysis, Including Some Turbidimetric and Nephelometric Methods. Vol. I. Theory, Instruments, pH. *Foster D. Snell* and *Cornelia T. Snell.* 3rd ed. 239 + xii pages. D. Van Nostrand Co., Inc., 250 Fourth Ave., New York 10, N. Y., 1948. Price $4.50.

This volume is the first of a trilogy intended to supersede a two-volume work published in 1936–37. It includes a brief treatment of the general theory supporting colorimetric methods of analysis, an extended survey of commercial devices, many of which differ by insignificant details, and a discussion of accuracy, sources of error, and

calculations, and proceeds to a moderately complete treatment of hydrogen-ion concentration and the theory and use of indicators.

The authors have formed this book by some rearranging of material but chiefly by inserting new words, sentences, and paragraphs within equivalent portions of the second edition. In most cases this has been more to amplify or clarify what may have seemed obscure in the second edition than to introduce developments of the past twelve years. These insertions are not always well assimilated. Beer's law as defined on pages 14 and 15 differs logically and importantly from Beer's law as defined on page 12. On page 14 the equation

$$-\log_e \frac{I_2}{I_1} = kCl$$

s derived, yet five pages later another writer is quoted as authority for the validity of the equation

$$I_2 = I_1 e^{-kCl}$$

Illustrations of apparatus tend to be poor. Fuzzy halftones of exteriors are generally employed rather than diagrams which would show functions.

The literature in this field is too voluminous for thorough survey in a book of this type but there are notable omissions. The use of a 1922 report as basis for a definition of terms while the abundant subsequent work of the Committee on Colorimetry of the Optical Society of America is neglected, the failure to notice any work on hydrogen-ion concentration by S. F. Acree or his associates subsequent to 1930, and the absence of any mention of the pH standards of the National Bureau of Standards will serve as examples. CHAS. P. SAYLOR

Organic Analytical Reagents. *Frank J. Welcher.* Vol. IV. xiii + 624 pages. D. Van Nostrand Co., Inc., 250 Fourth Ave., New York, N. Y., 1948. Price, $8 single volume; $7 series.

Volume IV of this useful treatise differs from its predecessors in being rather a compendium of miscellanea. Volumes I to III, because of their nature, were susceptible to much greater individual integration. Volume IV covers: acidic nitro compounds, arsonic acids, dithiocarbamates, xanthates, miscellaneous sulfur compounds, sulfonic acids, sulfinic acids, seleninic acids, alkaloids, diazonium compounds, carbohydrates, miscellaneous natural substances, miscellaneous compounds, lake-forming dyestuffs, hydroxyanthraquinone dyes, miscellaneous dyes, and dyes used in the detection of nitrite.

The treatment of the individual reagents is in the same form as in earlier volumes [reviewed in ANAL. CHEM., **20**, 88, 384 (1948)]. In the case of the dyes, Colour Index numbers are added to the usual Beilstein references.

The volume can be highly recommended to the research analyst and certainly testifies to the sedulous effort of the author of this series. G. FREDERICK SMITH

Practical Methods in Biochemistry. *Frederick C. Koch* and *Martin E. Hanke.* 5th ed. ix + 419 pages. Williams and Wilkins Company, Guilford & Mt. Royal Aves., Baltimore, Md., 1948. Price, $3.00.

In the main, this new edition is very similar to earlier ones. The authors have expanded the material on manometric methods, and have described some new or revised colorimetric methods. There is an extensive and detailed chapter on microbiological methods for assay of vitamins and amino acids.

In general, this is a manual of great value for students and for many investigators. One may hope that in future editions there will be some treatment of chromatographic methods, especially for separation of amino acids. Some experiments on biological oxidations and the enzyme systems concerned would also be a welcome addition. A discussion of some elements of spectrophotometry, especially Lambert's and Beer's laws, would add greatly to the student's understanding of the rational basis of colorimetry. The methods given for preparing nucleic acids and nucleoproteins (Chapter 4) involve treatment with strong alkali and yield degraded products; the modern methods, which employ much milder reagents for extractions, are greatly preferable. The formulation of the reaction of amino acids with formaldehyde (pages 45 and 139) in terms of the formation of a methylene derivative is now known to be incorrect; mono- and dimethylol derivatives are found, as indicated by the work of M. Levy and others.

These criticisms, however, do not seriously affect the positive merits of this book; it would be difficult to find a similarly extensive compilation of valuable analytical methods in biochemistry at so low a price. Professor Koch was active in the preparation of the manuscript and the correction of galley proof, so that this edition represents some of the latest work that he carried out before his death in January 1948. JOHN T. EDSALL

Mikromethoden zur Kennzeichnung organischer Stoffe und Stoffgemische. *Ludwig Kofler* and *Adelheld Kofler.* viii + 337 pages. 129 figures, 8 plates, and comprehensive tables. 17.2 × 24.8 cm. Universitätsverlag Wagner Ges. M.B.H., Innsbruck, Austria.

Procedures for identifying more than 1100 solids are based primarily upon the microdetermination of the melting points under the microscope. The melting points range from 25° to 340° C. Very little of the sample is required to make the test and the melting point is an excellent criterion of the purity. The index of refraction of the melt and the melting point of eutectics obtained by mixing the sample with certain pure substances are helpful indications. The characteristic appearance of the sample under the microscope is also noted, as well as the behavior of the sample during melting and sublimation. The methods described include the determination of the molecular weight and thermal analysis.

The theoretical basis of the work is explained very carefully and typical problems are suggested for work with students. The book should be in every up-to-date laboratory and library of organic chemistry. WILLIAM T. HALL

Calculations in Quantitative Analysis. *Philip W. West.* viii + 162 pages. Macmillan Co., New York, N. Y., 1948. Price, $2.75.

This book consists of eight chapters, presented in lecture style, of fundamental calculations in gravimetric and volumetric analysis. The last chapter contains a discussion of the correct use of significant figures, and the determination of the reliability of analytical results. The author introduces a number of problems which have a direct bearing on industrial applications from the fields of metallurgy, geology, and water analysis.

One of the important aims was to present the material in such a manner that the instructor might devote more time to lectures on chemical theories rather than to the problem part of the course in analytical chemistry. The author has been very successful in his aim, and has given clear explanations of the solutions of various types of problems involved in an elementary course. The problems at the ends of the chapters are well selected, and give the student an opportunity to test his knowledge of the principles explained. The appendix includes a handy graphic logarithm table.

WILLIAM J. TOMSICEK

The Electron Microscope. Its Development, Present Performance, and Future Possibilities. *D. Gabor.* viii + 164 pages. Chemical Publishing Co., Inc., Brooklyn 2, N. Y., 1948. Price, $4.75.

In this readable little book, Gabor gives a good introduction to the theory of the electron microscope, explains the basic principles involved in its operation, considers the present practical and theoretical limits, and devotes considerable space to speculating on possible improvements that may be expected in the future. The explanation of diffraction effects both by the specimen and by the lens aperture is one of the clearest this reviewer has ever encountered. The discussion on the structure of the electron microscope image is also excellently presented with the exception of his explanation of the effect of chromatic aberration on the image. Extensive but general descriptions of some commercial electron microscopes are included.

The most disappointing part of the book (very much misrepresented on the jacket) is the chapter on the achievements of the electron microscope, not so much because of its extreme brevity as its lack of consideration of the relationship of electron microscopy to

other scientific research. The biological applications are stressed, but the discussion is naive and misleading. The many uses of the electron microscope in chemistry, metallurgy, mineralogy, ceramics, etc., are practically ignored.

With the exception of a short chapter on the scanning microscope and on methods of analysis using the electron microscope, the remainder of the book is speculative in nature, discussing the ultimate limit of the electron microscope and methods by which it may be possible to improve the resolving power. A suggestion of the author's is given a very lengthy treatment which is completely out of character with the rest of the book. Included in this chapter is also a brief evaluation of the proton microscope, which appears to be careful and accurate.

The book concludes with an appendix of the diffraction theory of the electron microscope, in which the Abbe theory is discussed rather carefully with the modifications that must be made on applying it to the electron microscope.

This book is highly recommended to the reader who desires to gain an insight into the present status of research on the electron microscope without searching the very widespread literature. It is not recommended to the reader who is interested in evaluating the usefulness of the electron microscope in a specific research problem, nor to the microscopist who is interested in the manipulation of the instrument.

JAMES HILLIER

Synthetic Methods of Organic Chemistry. *W. Theilheimer.* Vol. I, 1942–44. x + 254 pages. Interscience Publishers, Inc., 215 Fourth Ave., New York 3, N. Y., 1948. Price $5.

This is an extremely accurate translation of Theilheimer's "Synthetische Methoden der Organischen Chemie," Repertorium I, in which the equations are identical to those appearing in the original German text. There are no significant deviations in the translation. A few errors have been introduced as, for example, in No. 23, page 7, where aminoacridone should read aminoacrine and where the *Chemical Abstracts* reference, which has been substituted for the *Chemische Zentralblatt* reference in the original, is wrong.

As the system employed is unique and not easily followed for the first time, this translation is of real value, especially to the beginning researcher in this field. This and the subsequent proposed volumes form a valuable adjunct to existing books on preparative organic chemistry. [The second volume in the German text ("Synthetische Methoden der Organischen Chemie," Repertorium II, by W. Theilheimer, S. Karger, Basel, Switzerland, and New York, N. Y., 1948) has recently been published.] It permits a rapid search to be made for possible modifications of older methods or new procedures for conducting a specified chemical transformation. This and subsequent volumes should be available for reference to every practicing organic chemist.

HARRY S. MOSHER

CORRESPONDENCE

Analysis of Cemented Carbide Compositions

SIR: While I have read with interest the article entitled "Analysis of Cemented Carbide Compositions" by Touhey and Redmond [ANAL. CHEM., 20, 202–6 (1948)], I find several important disagreements between the procedure described and the literature on the subject and its practical application to the analytical work on cemented carbide alloys in this laboratory.

1. Our tests show that molybdenum is precipitated to a large extent by cupferron in a tartaric acid solution and that it is completely precipitated in most other acid solutions. It is volatilized partially when ignited at temperatures over 525° C. when combined with tantalum, columbium, titanium, and tungsten. When present alone, it is extremely volatile at high temperatures. The authors show no data indicating that molybdenum was actually present in their test alloy.

2. In the paragraph starting "Fume filtrate II" it is suggested that an ignited precipitate containing oxides of titanium, columbium, tantalum, and iron be fused with potassium carbonate and the resulting solution filtered. The assumption thereafter seems to be that the tantalum and columbium will remain insoluble. This is contrary to our experience and to the published literature. A sodium carbonate fusion will cause the tantalum and columbium to remain insoluble, while a potassium carbonate fusion will result in solution of a large part of the tantalum and columbium oxides.

3. The authors suggest (page 203) that a solution containing cinchone and 10 ml. of nitric acid be evaporated to fumes of perchloric acid. Our experience has been that this evaporation is extremely hazardous. While danger of a perchloric acid explosion during oxidation of cinchonine probably depends on the exact technique employed during the evaporation, the only two accidents of this type in our laboratory occurred during the oxidation of cinchonine with nitric and perchloric acids. We have therefore discontinued attempting this particular oxidation.

Although we have made no analyses following the scheme of Touhey and Redmond, our experience with similar materials causes us to doubt seriously the soundness of the separations mentioned above.

W. C. BOWDEN, JR.

Ledoux and Co., Inc.
New York 13, N. Y.

SIR: The authors have carried out further tests following Mr. Bowden's criticism of the separation of titanium, tantalum, columbium, and iron from chromium and vanadium. The criticism is justified in that the separation is not satisfactory and the authors appreciate this being called to their attention.

The separation was provided (though inadvertently not so stated in the article) to cover only the very special case where chromium and vanadium are present. We know of no compositions now on the market which contain either element.

It was pointed out that the procedure is modified according to the elements known to be present, on the basis of spectrographic analysis or other information. Because these elements were known to be absent from the test mixtures, the separation under discussion was not used in obtaining the results shown in the paper.

Accordingly, the steps beginning on page 204, first full paragraph, lines 11 to 15, should be eliminated The following separation should be substituted when chromium and vanadium are present:

Fuse the ignited oxides of titanium, columbium, tantalum, and iron with 8 to 10 grams of C.P. potassium pyrosulfate and when the melt is cool, dissolve the melt in 50 ml. of water and 10 ml. of hydrochloric acid. Precipitate the titanium, columbium, tantalum, and iron by the addition of the solution to a dilute sodium hydroxide solution (10%). Add 1 gram of sodium peroxide, boil the solution for 3 minutes, and allow the precipitate to settle after the addition of ashless paper pulp. Filter the precipitate, using a No. 40 Whatman paper, and wash the precipitate well with a dilute sodium hydroxide solution.

The chromium and vanadium are then determined by the individual procedures given for these elements. The analysis of these complex materials is very difficult. There is much room for improved methods.

In connection with the point raised regarding evaporation, it should be noted that the solution is first reduced in volume before fuming. The reduction in volume is at low heat, under which conditions the cinchone is evidently sufficiently oxidized. In over 4 years of the use of this procedure no explosion or even violent reaction has occurred.

W. O. TOUHEY
J. C. REDMOND

Kennametal, Inc.
Latrobe, Pa.

Electron Microscope Society of America

F. A. HAMM, *General Aniline and Film Corp., Easton, Pa.*

THE 1948 meeting of the Electron Microscope Society of America was held in the McLennan Laboratory, Department of Physics, University of Toronto, Toronto, Canada, on September 9, 10, and 11. This meeting will be known as the E. F. Burton Memorial Meeting in memory of the late Professor E. F. Burton of the University of Toronto.

Approximately 175 people attended the meeting, which was held at the "birthplace" of electron microscopy in America. The Physics Laboratory was open for inspection, enabling those in attendance to see the electron microscopes built by Professor Burton and his students. Of special interest was the first magnetic electron microscope constructed in America, completed in 1938. It is a matter of record that the important status of electron microscopy in research today is largely the result of the pioneer work that emanated from the McLennan Laboratory. Although Professor Burton's death was most untimely, it is gratifying to know that he lived to see his former students lead the field in the application of electron microscopy to research in both industrial and university life.

The meeting was highlighted Friday night when all were invited to a complimentary dinner at the Hart House (campus) as guests of the University of Toronto. Perry C. Smith, incumbent president of the Electron Microscope Society of America, served as toastmaster and introduced the after-dinner speaker, Sidney E. Smith, president of the University of Toronto. The speaker's keen sense of humor was thoroughly enjoyed by all. F. O. Schmidt of the Massachusetts Institute of Technology then began his term as president of the society for the coming year.

After the dinner Robley C. Willams of the University of Michigan presented an invited paper on the "Electron Microscopy of Some Plant Viruses." He discussed some exploratory work on the growth, size, and division of tobacco mosaic and bushy stunt viruses.

G. David Scott of the University of Toronto deserves much credit for his efforts in serving as program chairman. The papers are listed below; only those papers of interest to the readers of ANALYTICAL CHEMISTRY are abstracted.

REPLICA TECHNIQUES

Comparison of Inorganic and Organic Replicas. C. J. CALBICK, Bell Telephone Laboratories, Murray Hill, N. J.

The sharply defined topography exhibited by sintered nickel-manganese powders was used to evaluate silica and collodion replicas from the standpoint of resolving power, strength, and stereoscopic interpretation. The silica replicas were better from all points of view.

An Evaluation of Several Types of Replicas. J. J. COMER AND F. A. HAMM, General Aniline and Film Corp., Easton, Pa.

The quality of the electron microscope image, from the standpoint of resolution and ease of interpretation, exhibited by the following four specimens was discussed: sample per se, chrome-shadowed sample, chrome-shadowed one-step silica replica, and chrome-shadowed two-step silica replica. The sample was a thin organic foil (exact nature not disclosed). The chrome-shadowed foil was the most reliable specimen; the shadowed one-step replica was better than the two-step silica replica.

A Method of Examination of Sections of Fine Metal Powders with the Electron Microscope. LAURENCE DELISLE, Sylvania Electric Products, Inc., Bayside, L. I., N. Y.

Small particles of tungsten, carbonyl iron, and carbonyl nickel were embedded in a thin film of organic resin. This sample was etched and polished according to standard metallographic practice. Formvar replicas of the exposed metal particles were then prepared. The polyhedral grain structure in tungsten and the "onion skin" ring structure of the carbonyl iron cross-sections could be resolved by this technique.

Electron Microscope Investigation of Opal Glass. THOMAS F. BATES AND MARY V. BLACK, Department of Earth Sciences, Pennsylvania State College, State College, Pa.

Commercial and laboratory samples of opal glass were etched with hydrofluoric acid and then collodion replicas (shadowed) were prepared. The size, structure, and growth of the constituent crystalline compounds can be evaluated. All the glasses showed a large number of small entrapped gas bubbles. The structures of the glasses were related to their composition and etching technique.

Some Uses of Uniform Sized Spherical Particles. R. C. BACKUS AND R. C. WILLIAMS, University of Michigan, Ann Arbor Mich.

Polystyrene latex, No. 580-G (Dow Chemical Co.) consists of a water suspension of spherical particles of remarkable uniformity of size (2540 ± 15 Å.). These particles can be shadowed and may be used to calibrate the electron microscope, to determine the shadowing angle in any localized region of the specimen, to measure the thickness of the shadowing metal, and to detect local distortion in the substrate.

Determination of Molecular Weights of High Polymers with the Electron Microscope. BENJAMIN M. SIEGEL AND HERMAN F. MARK, Polytechnic Institute of Brooklyn, Brooklyn, N. Y., AND DWIGHT H. JOHNSON, Princeton University, Princeton, N. J.

Several polystyrene fractions of known molecular weight (osmotic pressure) were deposited from extremely dilute solutions (cyclohexane) onto collodion substrates. The resultant particles are spheres and are assumed to be single molecules. From the volume of the single molecules, as measured in the electron microscope, the gram molecular weight can be calculated, assuming that the bulk density is the same as the density of the close-packed single molecule spheres. The values thus calculated agree well with the measurements from osmotic pressure. However, the technique is limited to very high (about 1,000,000) molecular weight polymers.

Ultrasonic Disintegration of Cellulose Fibers before and after Acid Hydrolysis. F. F. MOREHEAD, American Viscose Corp. Marcus Hook, Pa.

Ultrasonic (700 kc.) vibrations when applied to natural and synthetic fibers before and after mild acid hydrolysis cause the fibers to disintegrate into a distinct fibrillar structure. The length of these fibrils depends upon the orientation and crystallinity of the original fiber. Regenerated fibers give rise to shorter and thinner fibrils (crystallites). These fibril sizes were related to the structure and the various physical properties of the original fibers.

SYMPOSIUM ON REPLICA TECHNIQUES

R. D. Heidenreich, Bell Telephone Laboratories, Murray Hill, N. J., spoke on the oxide and polystyrene-silica techniques. The anodic oxide type of replica appears to be best for metals, although this type of replica cannot be shadow-cast. For some unknown reason, shadowing oxide replicas impairs their quality. He pointed out the need for carefully preparing the surface before attempting to prepare the replica. A new type of "negative" resin replica was described in which methyl methacrylate monomer (stored cold) was allowed to polymerize and set up hard as the solvent evaporated. The tacky polymer is squeezed against the surface to be replicated just before hardening. Silica is then evaporated onto this negative replica in order to make a positive. Mention was made that the silica, after condensation, may in some cases migrate 0.5μ.

R. C. Williams, Department of Physics, University of Michigan, Ann Arbor, Mich., discussed the pros and cons of using the collodion (Formvar, Parlodion) technique. He felt that shadow-cast collodion replicas would resolve better than 100 Å. despite popular beliefs. The topography of the original surface determines the required thickness of the collodion replica. For smooth surfaces, the extremely thin (100 Å.) replicas may be strengthened by vaporizing silicon monoxide or beryllium on the back side, or even over the shadow-cast side of the thin film.

The transfer replica technique was reviewed. In this technique a metal (platinum, palladium) is vaporized onto the sample supported on glass. This metal film is then backed up with collodion. After the specimen screens are protected with paper the replica is stripped from the glass with Scotch tape. The specimen areas are punched out, the paper is removed, and the replica is ready for examination.

A negative resin–positive resin replica process may be carried out by embedding the original sample in softened polyethylene. This negative resin replica is insoluble in amyl acetate, so that a Formvar solution may be cast onto it, thereby forming the positive replica.

Mary S. Jaffe, Lamp Department, General Electric Co., Cleveland, Ohio, described some of her techniques in handling and washing fragile replicas. Naphthalene used in conjunction with the replica on the specimen screen prevents the replicas (organic) from tearing as they dry. The naphthalene is later removed by sublimation.

James Hillier, RCA Laboratories, Princeton, N. J., spoke on miscellaneous techniques and applications. He pointed out the inadequacy of spot checks, and the lack of replicating minor changes in the appearance of the sample. Although the effects of high vacuum must always be considered, apparently these effects are very minor if the sample (bacterium) has been well dried previously. It was also suggested that a thin film of a water-soluble salt (sodium chloride) be evaporated onto the sample prior to the silica or the metal. This facilitates removal of the replica in water. The great depth of field of the electron microscope is of aid to those interested in metallography; in the past the use of the metallograph has necessitated the removal of most of the surface elevations because of the narrow depth of field.

BIOLOGICAL APPLICATIONS

The Friday morning session was devoted entirely to biological applications.

A Study of Bacterial Flagellation with the Delft Electron Microscope. A. L. Houwink, Technical Physics Department, Delft, Netherlands, communicated by Woutera van Iterson, National Institute of Health, Bethesda, Md.

Electron Microscopy of Mycobacteria Tuberculosis. E. M. Brieger, Papworth and Strangeways Laboratories, Cambridge, and V. E. Cosslett, Cavendish Laboratory, Cambridge, England.

Possibility of Demonstrating an Intermediate Level of Structure in Normal Bacteria. James Hillier, RCA Laboratories, Princeton, N. J.

Presence of Dense Particles in the Neurotubules of Nerves Infected with Poliomyelitis Virus. E. DeRobertis and F. O. Schmidt, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

Quantitative Virus Counts with the Electron Microscope. D. Gordon Sharp, School of Medicine, Duke University, Durham, N. C.

The Ultrastructure of the Retinal Rods of the Guinea Pig Eye. Fritiof S. Sjöstrand, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

Morphology of Nucleoprotein of Spinal Cord as Revealed by Electron Microscopy. Joseph T. Melnick, Yale University School of Medicine, New Haven, Conn.

Observations on Chromosome Structure in Resting Cells with the Electron Microscope. Benjamin M. Siegel, Fernando Calvet, and Kurt G. Stern, Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

Fibrillar Structure in Rat Fibroblasts as Seen by Electron Microscopy. F. B. Bang and G. O. Gey, The Johns Hopkins Hospital, Baltimore, Md.

Replica Studies of Collagen Fibers. J. Gross, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

THIN SECTIONS

The next session included papers devoted to thin sections of a variety of materials.

Sectioning Techniques for the Electron Microscope Using a Conventional Microtome. Richard F. Baker and Daniel C. Pease, Departments of Anatomy and Experimental Medicine, University of Southern California, Los Angeles, Calif.

The sectioning of biological materials to give specimens sufficiently thin for the electron microscope presents a real challenge. The technique described in this paper appears to fulfill most of the requirements. A rotary hand-operated microtome was used; the speed of cutting was of no significance. The original fixation of the tissue plus the subsequent embedding (about 12 steps) requires from 1 to 2 weeks. Apparently the most serious artifacts in any such technique are caused by the original fixation. Sections of liver tissue, rat muscle, rat small intestine, and chromosomes were illustrated. In general, the procedure is simply a carefully thought out modification of old established sectioning techniques.

A New Method of Sectioning Single Filaments of Synthetic Fibers for the Electron Microscope. Marshall D. Earle and Jean A. Minkin, Franklin Institute, Philadelphia, Pa.

Although high quality cross sections of textile fibers for examination in the electron microscope have not yet been prepared, the investigation disclosed in this paper is certainly a step in the right direction. High speed ultramicrotome sections of rayon (embedded in low melting paraffin) were illustrated. However, the yield of this technique is very poor and usually only fragments of cross sections were obtained.

Preparation of Human Cardiac Muscle for Electron Microscope. W. E. Adolph, Department of Investigative Medicine, Birmingham General Hospital, Van Nuys, Calif., and R. F. Baker, School of Medicine, University of Southern California, Los Angeles, Calif.

Thin Metal Sections for Electron Microscopy and Electron Diffraction. R. D. Heidenreich, Bell Telephone Laboratories, Inc., Murray Hill, N. J.

The technique described in this paper is a new approach to the preparation of very thin specimens of metals. The specimens are not mechanically deformed, and yet they (of themselves) are thin enough to be used in the electron microscope. Thin (0.005 inch) disks of aluminum and aluminum-copper alloys were made thinner by electrolytic (anodic) action. Only one side of the metal disk is allowed to react at a time. Both sides are electropolished in this way until a very small opening begins to form at the center. Electrolysis is then immediately stopped. The electron microscope image of this type of specimen results mostly from diffraction, so that dislocations and crystal planes may be identified.

INSTRUMENTATION

The Saturday morning session was devoted to instrumentation.

The Philips Electron Microscope. A. C. van Dorsten, J. B. LePoole, and A. Verhoeff, read by W. J. Oosterkamp, Institute of Technology, Delft, Holland.

This new electromagnetic electron microscope was developed at the Institute for Electron Microscopy of the Institute of

Technology at Delft. There appear to be three noteworthy features: a variable accelerating voltage from 40 to 100 kv., a theoretical magnification up to 80,000×, and a variable adjustment system of apertures for diffraction and microscopy. Certain specific areas in the specimen may be used for diffraction by making a few changes in the aperture system. The image is recorded on roll film.

Phase Contrast in Electron Microscope Images. E. G. RAMBERG, RCA Laboratories, Princeton, N. J.

The intensity distribution in the focused image of a very thin organic film was treated theoretically from the standpoint of phase delays impressed by the film on the incident parallel electron rays. The relation between contrast and phase delay was outlined. The results of these calculations were compared with the practical observations discussed in the paper, "The Magnetic Electron Microscope Objective. Contour Phenomena and the Attainment of High Resolving Powers," by James Hillier and E. G. Ramberg [*J. Applied Phys.*, **18**, 48–71 (1947)].

Artifacts in Electron Microscopy. J. J. KELSCH, Interchemical Corporation, New York, N. Y.

This paper consisted of a survey of the common artifacts that may appear in everyday electron microscopy. Heat effects, in the microscope, sublimation in the microscope, desiccation and contamination arising in the microscope from the sample itself or from the gun filament, were considered.

The Illuminating System of the Electron Microscope. JAMES HILLIER AND S. G. ELLIS, RCA Laboratories, Princeton, N. J.

With the advent of the self-biased gun, it became desirable to gain data on the angular aperture of illumination, the distribution of the illumination at the specimen, and the total current reaching the specimen. These data were illustrated and compared with the zero bias gun. The advantages of the self-biased gun were again enumerated. The chief disadvantage of the self-biased gun appears to be the contamination of the specimen because of the higher illumination intensity. The 20-fold increase in illumination and the smaller angular aperture of illumination for a given condenser setting with their resultant advantages were stressed.

Auxiliary Supporting Nets for Fragile Electron Microscope Specimens. MARY S. JAFFE, Lamp Department, General Electric Co., Cleveland, Ohio.

Heavy particles tend to tear thin organic substrates and other types of specimens may move under the electron beam. The technique for preparing a very porous substrate "net" for holding these specimens was described. The strength of these resin nets was illustrated by micrographs of smokes, dusts, and centrifuged suspensions caught in the net.

Adjustment and Manipulation of the Electron Microscope. JAMES HILLIER, RCA Laboratories, Princeton, N. J.

There has been much need for a paper of this nature. The average electron microscopist in the field may not have the time or the technical background to examine critically the sundry possibilities that exist in the cleaning, adjustment, and manipulation of the electron microscope. This paper very adequately provided the methods for properly carrying out these procedures. Virtually nothing was overlooked. A few of the details discussed were specimen stability, lens aberrations, vibration, stray magnetic fields, alignment, and cleanliness.

MISCELLANEOUS

Diffraction Microscopy. D. GABOR, British Thomson-Houston Co., Research Laboratories, Rugby, Warwickshire, England, presented by F. W. CUCKOW, Royal Cancer Hospital, London.

This technique involves a two-step rather unconventional procedure for gaining images of very high resolution. The first step will employ electrons emanating from a very small source [or small effective (aperture) source] and forming a "diffraction diagram" after coherently diverging through the specimen. This diffraction diagram (no likeness to the original) is then illuminated with visible light which is analogous to the original electronic illumination (but larger by a factor of the ratio of the wave lengths). An image of the original sample is thusly reconstructed. To date only light waves have been used for both steps; however, research using electrons for the first step is in progress.

An Experimental 400-Kvolt Electron Microscope. A. C. VAN DORSTEN AND J. B. LEPOOLE, read by W. J. OOSTERKAMP.

The electron microscopical examination of relatively thick (1 to 2 microns) biological specimens necessitates the use of higher energy electrons. This is accomplished by employing higher accelerating voltages. However, the stability of the high voltage and shielding from x-rays are important problems. The instrument in the Phillips Research Laboratory appears to have fulfilled the requirements. The lesser transfer of energy to the specimen is also of some significance.

A Study of the Simultaneous Electron and Molecular Bombardment of Electron Microscope Specimens. JAMES HILLIER, RCA Laboratories, Princeton, N. J.

What has been arbitrarily called specimen contamination may in some cases be a removal of some earlier contamination or an etching of the specimen. In this study gases and vapors were admitted to the specimen chamber and directed onto the specimen. The specimen was simultaneously illuminated with the electron beam. Some vapors (helium, benzene, and carbon tetrachloride) have no effect. Water vapor, on the other hand, causes a serious effect. Presumably the thermal energy transferred when the electrons suffer inelastic scattering may cause specimen to undergo a chemical reaction. A loss of 20 electron volts is equivalent to 225,000° C. per electron.

Further Researches on the Electron Microanalyzer. S. G. ELLIS, RCA Laboratories, Princeton, N. J.

The use of the electron microanalyzer for detecting trace amounts of chemical elements has been described by James Hillier ["Microanalysis by Electrons," *Phys. Rev.*, **64**, 318, 319, (1943)]. However, this investigation has shown that the technique has certain limitations—for example, from 10^{-11} to 10^{-12} gram of the element can be detected, but this element must cover about 10% of the area illuminated by the probe. Smaller regions on the specimen could be probed if a further reduced image of the electron source—i.e., illuminating probe—were used. However, under these conditions the intensity of illumination becomes so great that the contamination of the sample becomes prohibitive.

Observations of Carbon Crystal Structure. J. D. BOADWAY, Shawinigan Chemicals, Ltd., Shawinigan Falls, Quebec.

This paper demonstrated the usefulness of electron microscopy in particle size studies, where diffraction data might not accurately represent the sample.

The Growth of Colloidal Crystals. JOHN H. L. WATSON, The Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.

The growth of submicroscopic colloidal crystals (tungstic oxide, iron oxide, vanadium pentoxide) was followed with the electron microscope. The effects of time, concentration, washings, etc., were observed and theories related to the kinetics of growth were postulated.

An Electron Microscope Technique for the Study of Polymeric Molecules. WILBUR KAYE, Tennessee Eastman Corp., Kingsport, Tenn.

The essence of this paper was a discussion of a special technique for mounting polymeric molecules. The polymeric material is deposited on a substrate of aluminum-beryllium alloy, which in turn had been vaporized onto a hydrophilic layer (surface active agents, salts, sugars, etc.). This latter layer facilitates the easy stripping of the specimen from a glass plate under water. The supporting substrate exhibits very little structure.

The Effect of Shadowing, by Metallic Evaporation, upon Determination of Particle Size. H. KAHLER AND B. J. LLOYD, JR., National Cancer Institute, Bethesda, Md.

It was demonstrated that measurements of particle (virus) dimensions made on shadow-cast specimens may lead to errors because of the increase in the apparent diameter of the particle. A detailed study of the way in which metal granulates and accumulates on particles was made.

Particle Size Correlation with X-Ray Methods. K. L. YUDOWITCH, Department of Physics, University of Missouri, Columbia, Mo.

The electron microscope is especially useful in the study of particle size distribution in the range above 100 Å. As the particle size increases beyond this value, the x-ray line broadening method becomes increasingly inaccurate. However, equations were derived for use with x-rays of very long wave length permitting more accurate measurements on particles up to a micron. These data for colloidal gold were checked against electron microscope measurements.

Electron Microscope Studies on the Structure of Larger Animal Viruses. PIERRE LEPINE, Viruses Division, Pasteur Institute, France (at present at Institute of Microbiology, University of Montreal).

Third National Instrument Conference and Exhibit

RALPH H. MÜLLER, *Contributing Editor*

THIS important conference and exhibit was sponsored by the Instrument Society of America and met jointly with divisions of the American Society of Mechanical Engineers, the American Institute of Physics, and the American Institute of Electrical Engineers. The meetings were held in Convention Hall, Philadelphia, Pa., September 13 to 17. More than 12,000 registrants attended and this impressive figure truly reflects the interest and importance of instrumentation because the general public was not admitted.

More than 150 exhibits occupied 200 booths in which practically every type of industrial and scientific instrument was available for demonstration and detailed examination. Some outstanding impressions gained from countless interesting items were the Librascope mechanical computer elements at the Askania exhibit; the uniform excellence of the General Electric exhibit; the Leeds & Northrup 140-point Speedomax recorder and the microvolt recorder; the Milton Roy proportioning pumps for automatic continuous titrations; the National Bureau of Standards magnetic fluid clutch; and Perkin-Elmer Corp.'s electrophoresis apparatus.

The conference activities included carefully planned lectures for instrument technicians, numerous educational films by instrument companies, and meetings of committees of the cooperating societies. Formal papers read at the various technical sessions are listed here with title, author, and author's affiliation.

Comparison of Small and Medium Electric Power Servomotors. ROBERT S. EDWARDS, Sperry Gyroscope Co., Great Neck, L. I.

Photographic Instrumentation Used in the Development of the Bat Missile. H. K. SKRAMSTAD, Guided Missiles Section, Ordnance Development Laboratory, Washington, D. C.

Temperature and Pressure Measurements in Rockets. R. J. HAVENS, Naval Research Laboratory, Washington, D. C.

Demonstration of Automatic Control Principles. GERALD F. AKINS, assisted by JOHN H. KOWALSKI, Eastman Kodak Co., Rochester, N. Y.

Valve Characteristics and Automatic Control. J. G. ZIEGLER AND N. B. NICHOLS, Taylor Instrument Cos., Rochester, N. Y.

Control Valve Body Design. D. P. ECKMAN, Cornell University, Ithaca, N. Y., and R. B. WEREY, Conoflow Corp., Philadelphia, Pa.

Liquid Flow Characteristics of a Pipe Line and a Control Valve. OTTO KNEISEL, Hammel-Dahl Co., Providence, R. I.

Looking into the Future of Electronics. GORDON VOLKENANT, Minneapolis, Minn.

Use of Interconnected Control Instrumentation for Offsetting Unfavorable System Characteristics. J. A. PELLETTERE, Gulf Oil Corp., Pittsburgh, Pa.

Experimental Application of Combustion Controls to a Process Heater. W. E. BOYLE, Shell Oil Co., Inc., Wood River, Ill., AND P. R. HOYT, Shell Development Co., San Francisco, Calif.

The ABC's of Multi-Element Control. C. H. BERNARD, Bailey Meter Co., Cleveland, Ohio.

Supervisory Control Systems. LOUIS GESS AND R. M. HUTCHINSON, Brown Instrument Co., Philadelphia, Pa.

Pneumatic Transmission Time Lags. MEAD BRADNER, Foxboro Co., Foxboro, Mass.

Instrumentation by and with Controlled Volume Pumps. ROBERT T. SHEEN, Milton Roy Co., Philadelphia, Pa.

Science in Crime Detection. L. V. BOARDMAN, Federal Bureau of Investigation, Philadelphia, Pa.

Flow Measurement of Gases and Liquids through Primary Elements in Pipe Sizes of Less than 2 Inches. H. W. STOLL, Taylor Instrument Cos., Rochester, N. Y.

Organized Instrument Engineering. J. JOHNSTON, JR., E. I. du Pont de Nemours & Co., Wilmington, Del.

Development of Instrument Curricula. M. B. HALL, Foxboro Co., Foxboro, Mass.

Response Characteristics of Resistance Thermometers. A. J. HORNFECK, Bailey Meter Co., Cleveland, Ohio.

Bourdon Tubes in 5000 p.s.i. Pressure Transmitters. O. C. BREWSTER, Litchfield, Conn.

Laboratory Analogs for Electric Furnaces. S. B. HIGGINS AND R. M. HUTCHINSON, Brown Instrument Co., Philadelphia, Pa.

Fractionation Instrumentation and Control. D. M. BOYD, JR., Universal Oil Products Co., Chicago, Ill.

Functional Flexibility in Process Control. F. H. TRAPNELL, E. I. du Pont de Nemours & Co., Wilmington, Del.

Electron Microscopy. JAMES HILLIER, Radio Corp. of America, Princeton, N. J.

Measurement of the Particle Size of Sub-sieve Powders. R. E. PAYNE, Sharples Corp., Philadelphia, Pa.

Infrared Instrumentation. VAN ZANDT WILLIAMS, American Cyanamid Co., Stamford, Conn.

Physical Principles of Vacuum Measurements and Production. C. H. BACHMAN, Syracuse University, Syracuse, N. Y.

Acoustics Instrumentation. R. H. BOLT, Massachusetts Institute of Technology, Cambridge, Mass., AND R. K. COOK, National Bureau of Standards, Washington, D. C.

Tools of the Physics Teacher. R. M. SUTTON, Haverford College, Haverford, Pa.

Radio Spectroscopy. C. H. TOWNES, Columbia University, New York, N. Y.

Gas Analysis by the Mass Spectrometer. A. O. C. NIER, University of Minnesota, Minneapolis, Minn.

Radioactive Tracer Techniques and Measurements. L. F. CURTISS, National Bureau of Standards, Washington, D. C.

Fail-Safe Operation of Electronic Circuits. G. D. HANCHETT, R.C.A. Mfg. Co., Camden, N. J.

Cathode Ray Oscillograph Developments for Laboratory and Production Use. C. BERKLEY, Allen B. DuMont Laboratories, Inc., Passaic, N. J.

Oil Film Thickness Indicator for Journal Bearings. M. L. GREENOUGH, National Bureau of Standards, Washington, D. C.

Résumé of A.I.E.E. Conference on Electron Tubes for Instrumentation and Industrial Use Held in Philadelphia, March 29 and 30. W. R. CLARK, Chairman, A.I.E.E. Joint Subcommittee on Electronic Instruments.

A Study of Slide Wire Contact Resistances. W. E. BELCHER, JR., Brown Instrument Co., Philadelphia, Pa.

Developments of Self-Balancing Recorders. A. J. WILLIAMS, JR., Leeds & Northrup Co., Philadelphia, Pa.

Salinity-Temperature-Depth Recorder. A. W. JACOBSON, Bristol Co., Waterbury, Conn.

Methods for Analysis of Steel

The British Standards Institution, Sales Department, 24 Victoria St., London, S.W. 1, England, has issued methods B.S. 1121 for the analysis of steel: Part 7. Tin in pig iron, plain carbon steels, and certain low-alloy steels. Part 8. Chromium present in small amounts in carbon and low-alloy steels. Part 9. Phosphorus in high chromium-nickel steels. Part 10. Silicon in all types of irons and plain alloy steels other than high tungsten and high tungsten–molybdenum steels. Part 11. Carbon in steel and low-carbon ferrochromium.

Copies are available from the institution at 1 shilling each.

International Congress on Analytical Chemistry

A. H. W. ATEN, *Hilversum, Netherlands*

AN INTERNATIONAL congress on analytical chemistry was held at Utrecht (Netherlands) on June 1, 2, and 3, 1948, under the auspices of the Netherlands Chemical Society and with the support of the Dutch chemical industry. The purpose of this congress was to provide information for industrial chemists about recent analytical developments, an aim which was facilitated by ample opportunities for personal contacts, during these days. Special attention was given to the organization of analytical work in industrial laboratories and to the training of analytical chemists.

Approximately 350 people were present, among whom about 90 had come from other countries in Western Europe. Great Britain was represented by some 40 visitors. It was much regretted that no participants from the United States attended. I. M. Kolthoff of Minneapolis and J. J. Lingane of Harvard University had submitted reports, which were very much appreciated and were discussed with a great deal of interest. The congress was seriously disappointed by the fact that J. Heyrovský of Prague, who had promised to lecture about new developments in polarography, had been refused permission to leave Czechoslovakia by the new government.

Apart from a few general speeches, the activities of the congress were divided over the following five sections: General Methods and Normalization, Electrical Methods, Emission Spectrography, Optical Methods and Physical Methods of Separation, and Microbiological Methods and Detection of Traces. In each section one or two lectures were given by invited speakers from outside Holland. After those a number of reports were discussed. These papers had been sent to the members of the congress as preprints, and it was assumed that everybody present was already familiar with them.

After the rector of the university and the president of the Netherlands Chemical Society had both spoken a few words of welcome and after a telegram from the Australian Microchemical Society had been read, C. J. van Nieuwenburg, professor of analytical chemistry at the Delft Institute of Technology and president of congress, delivered the opening speech. He described the development of analytical chemistry and traced its borders. Special attention was paid to the difference between analytical chemistry as a science and chemical analysis as a routine technique.

In the course of the congress several general lectures were given, the first of which by G. Charlot (Paris) dealt with the duties of the present-day analytical engineer. The necessity was stressed of adapting analytical procedures to the ability of unskilled personnel. Application of chemical theory is very profitable, as illustrated by the separation of columbium from tantalum and by the use of silver in the presence of chloride ion and of gold in cyanide solution as reducing agents.

The time reserved for the lecture of Heyrovský was filled by Professor Duval (Paris), who spoke about analysis of mixtures by decomposition. A special apparatus, a "thermobalance," was described, by which mixtures of calcium and magnesium are analyzed by decomposition of the oxalates and copper-silver mixtures by decomposition of the nitrates.

In the final session H. W. Thompson (Oxford) delivered an enthusiastic lecture on the use of infrared spectrography. Technical improvements involving synthetic crystals were discussed and beautiful results were shown, which had been obtained in the analysis of organic compounds. Everybody regretted that time did not permit the speaker to continue with a review of applications of Raman spectrography.

In the first section E. C. Wood (London) lectured on recent applications of statistics to chemical analysis. Expert advice by statisticians may often save enormous expense. Thus a detailed mathematical study was the cause of an important simplification of the regulations for sampling sugar in bags, which is imported into the United States. An interesting example showed how statistical treatment may discover inaccuracies in analytical procedures.

W. J. Gooderham (London) gave a most impressive demonstration of a new method for gas analysis, depending on volumetric measurements using soap membranes moving through gas pipets. The entire analysis of illuminating gas is carried out in a single train of absorbers.

Reports by Pieters and Schmidt and by Forbes dealt with standardization of analytical methods, one by Degens with the task of the analytical chemist in industry, and one by Boeke with the economic aspects of the rationalization of analysis.

Aten junior had submitted a paper on the use of isotopic tracers in analytical chemistry, Kistemaker one on the mass spectrometer and some of its analytical applications, Gouverneur a paper on organic elemental analysis, and Deinum and Dam one about a modified Orsat apparatus for the analysis of purified coke-oven gas.

In the section for electrical methods E. Leclerc (Liége) spoke about electrical methods for analysis in industry. Speed, sensitivity, and precision of electrical measurement techniques were discussed. Application of these methods in metallurgy, in the oil industry, and in water purification was mentioned. The same techniques are useful in some types of organic analysis, as in the determinations of vitamins. Application to the detection of rare elements in fertilizers may also be considered.

Among the reports offered were two on water analysis, by Bijker and Janssen. Claassen described a continuous reading vacuum tube voltmeter for electrometric titrations. Lingane had written about controlled-potential electroanalysis and Kolthoff about amperometric titrations. Applications of the polarographic method to the determination of alkali metals, calcium, barium, aluminum, lead, and zinc in glass and of lead, cadmium, and zinc in metals and in ores were treated by Vandenbosch and by Favre.

In the spectrographic section the main lecture was delivered by E. Loeuille (Paris), who presented a very extensive treatment of the construction of prism spectrographs, the use of photographic plates, and the principles of qualitative and quantitative spectrographic analysis. Inaccuracies in current wave-length tables were reported. The methods of Gerlach and of Kayser for the analysis of alloys were discussed critically. The use of a hyperbolic rotating sector was mentioned.

Milbourn had submitted a paper on factors affecting the accuracy of spectrographic analysis and D. M. Smith with G. M. Wiggins had written about improvements in the technique of spectrographic analysis of high purity materials. Spectrographic analysis after preliminary concentration was the subject of communications by R. L. Mitchell with R. O. Scott and by Sempels. Castro with Phéline described the determination of aluminum, and Herman the determination of columbium and tantalum. J. Orsag dealt with the determination of sodium in aluminum.

In the fourth section G. Duyckaerts (Liége) spoke about absorption spectra and their analytical applications. He gave many examples of chromophoric groups and stressed the necessity of collecting extensive data on absorption spectra for analytical purposes. He mentioned the sensitivity of absorption spectra to traces of many impurities. Thus 0.1% of cyclohexanone in cyclohexane gives rise to a characteristic band. Special attention is given to the important problem of deviations from Beer's law. Molecular changes or molecular interactions may play a part; theoretically the absorption must vary with the refractive index of the mixture and—most important of all—deviations are to be expected if the wave-length band used is wider than the absorption band. For this reason use of interference filters is desirable.